

CHLAMYDIAL SALPINGITIS

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To my daughters
Johanna and Emma
- for their patience



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ORIGINAL PAPERS

This thesis is based on the following publications, which will be referred to in the text by their Roman numerals:

- I. Mårdh P-A, Ripa T, Svensson L and Weström L: Chlamydia trachomatis infection in patients with acute salpingitis. N Engl J Med 296: 1377-1379, 1977.
- II. Møller BR, Weström L, Ahrons S, Ripa KT, Svensson L, von Mecklenburg C, Henrikson H and Mårdh P-A: Chlamydia trachomatis infections of the Fallopian tubes. Histological findings in two patients. Br J Vener Dis 55:422-428, 1979.
- III. Mårdh P-A, Lind I, Svensson L, Weström L and Møller BR: Antibodies to Chlamydia trachomatis, Mycoplasma hominis and Neisseria gonorrhoeae in sera from patients with acute salpingitis. Br J Vener Dis 57: 125-129, 1981.
- IV. Svensson L, Weström L, Ripa KT and Mårdh P-A: Differences in some clinical and laboratory parameters in acute salpingitis related to culture and serological findings. Am J Obstet Gynecol 138:1017-1021, 1980.
- V. Svensson L, Weström L and Mårdh P-A: Acute salpingitis with Chlamydia trachomatis isolated from fallopian tubes - clinical, cultural and serological findings. Sex Transm Dis 8:51-55, 1981.
- VI. Svensson L, Mårdh P-A and Weström L: Infertility after acute salpingitis - with special reference to Chlamydia trachomatis associated infections. (Submitted)

ABBREVIATIONS AND DEFINITIONS

AS	= Acute salpingitis, visually confirmed
C-AS	= Acute salpingitis, linked to <u>Chlamydia trachomatis</u> by isolation and/or results of serological tests
G-AS	= Acute salpingitis, linked to <u>Neisseria gonorrhoeae</u> by isolation and/or serological tests
CG-AS	= Acute salpingitis, linked to both <u>C trachomatis</u> and <u>N gonorrhoeae</u> by isolation and/or results of serological tests
NCNG-AS	= Acute salpingitis, linked neither to <u>C trachomatis</u> nor to <u>N gonorrhoeae</u>
PID	= Pelvic inflammatory disease (as defined by the investigators cited). Generally synonymously with AS
LGTI	= Lower genital tract infection
STD	= Sexually transmitted disease
NGU	= Nongonococcal urethritis
IHA	= Indirect haemagglutination
MIF	= Micro-immunofluorescence
OC	= Oral contraceptive
IUD	= Intrauterine device

INTRODUCTION

General aspects of acute non-tuberculous salpingitis

Acute non-tuberculous salpingitis (AS) is a disease that mainly afflicts women in their reproductive years (186).

The diagnosis of AS is, in most parts of the world, based on clinical signs and symptoms and certain laboratory data. The reliability of clinical diagnosis based on such signs and symptoms has varied between 46 and 89 % when verified by the routine use of laparoscopy (6,18,51,71-74,80,84,163,188). For the description of infectious conditions in the upper female genital tract and pelvic cavity the term pelvic inflammatory disease (PID) is widely used. The criteria for the diagnosis of PID are not uniform, which makes the comparison of findings in different series difficult (47).

Estimates from Sweden have arrived at 11,000 to 17,000 AS-cases annually (102). The annual number of PID cases in the United States during the 1970's has been estimated to be between 505,800 and 850,000 (21,33,157,158,177).

In England and Wales, the admission of PID cases to hospitals rose by 50 % from 1968 to 1977, and reached in 1977 10,960 (2).

There is general agreement that, in the industrialized countries, the annual peak incidence of PID per 1,000 women is in the age group 20-24 years, whereas sexually active teenagers have the highest relative risk of acquiring the disease (154,186). The age-specific rates for PID in two widely different populations, i.e. Atlanta, Georgia, USA, and Lund, Sweden, were strikingly similar for the year 1965 (186). The age-specific figures of PID published from the UK (1) are in agreement with those from Sweden and the United States (186).

There is a consensus of opinion that AS is caused, most often, by micro-organisms which ascend from the lower genital tract (15,187). Only in a small proportion of the AS cases in women of a fertile age, is the infection secondary to inflammatory processes affecting organs outside the genital tract, usually the appendix. In a series from Lund, Sweden, secondary AS has been found < 1 % of the cases (186). Haematogenous spread of micro-organisms to the fallopian tubes is a rarity (63,130).

From the lower genital tract, there are two possible modes of spread to the fallopian tubes, i.e. through the parametria via lymphatic channels, or canalicular via the endometrial cavity. In the majority of AS cases, the canalicular mode of spread is the most probable. Observations supporting this statement are: A. In the vast majority of laparoscopically-verified AS cases, signs of an infection were also present in the lower genital tract (187);

B. Isolation of aetiological agents of AS, not only from the tubes, but also from the endometrium (56,101,161,193); C. After cornual resection of the tubes, no recurrence was found in over 1,000 cases of AS, which were followed for up to 8 years (35), while the recurrence rates in two long-term Swedish studies of AS patients, conservatively treated, were 13 and 23 % respectively (36,184). In those studies the numbers of patients were 283 and 415 respectively, and the follow-up time $2\frac{1}{2}$ to 5 years and 6 to 14 years respectively (36,184); D. Ligation of the isthmus of the tubes prevents salpingitis in animals experimentally infected with agents supposed to be common causes of AS in humans (104,107).

AS is closely correlated to sexual activity. This is evident, i.a. from the following facts: acute non-tuberculous PID rarely occurs in nuns, except secondarily to appendicitis (156); women with PID are only exceptionally virgins (191).

Moreover, there is a close relationship between the epidemiology of the sexually transmitted diseases (STD) and the epidemiology of PID (1,15,89,186).

Etiological agents in acute salpingitis

Of the etiological agents of AS, Neisseria gonorrhoeae was the first to be isolated from the fallopian tubes (183). This organism is also the most extensively studied. The isolation rate of gonococci from the lower genital tract in women with PID, has been found to vary between 6 and 80 % (47,159, 171). In women harbouring N gonorrhoeae in the lower genital tract, the organism has been recovered from peritoneal fluid in 6 to 83 % of the patients with PID (159,171). In most of these studies, the sampling was made via culdocentesis. Discrepancies have, however, been reported between the culture results obtained from the cul-de-sac samples and samples obtained directly from the tubes (162,163). Therefore, the acquisition of specimens for isolation studies from the cul-de-sac, either during laparoscopy or via culdocentesis has been questioned (162,163). In specimens obtained directly from the fallopian tubes, N gonorrhoeae was recovered in 0 % (42), 6 % (71), 13 % (185) and 42 % (162) of women culture-positive for gonococci from the lower genital tract.

The complication rate in women with gonorrhoea, in this context the rate of PID, in Sweden during the 1960's was estimated to be 10 to 15 % (41,82). The diagnosis of PID in these studies was based on clinical criteria and without considering possible double infections, e.g. C. trachomatis (41,82). The relative complication rates seem to have decreased to less than 10 % during

the 1970's (38,186). These cases of AS were, however, laparoscopically verified, but double infections with gonococci and chlamydiae were neither in these investigations excluded (38,186). Differences in the complication rate might be related to the relative prevalence in various areas of gonococcal strains of different auxotypes, some of which might be more closely related to AS (68).

Several studies have suggested that N gonorrhoeae "paves the way" for a super-infection by other micro-organisms, especially with anaerobes (for reviews see ref. 47,68,95,160).

Yet another sexually transmitted micro-organism, which has been associated with AS, is Mycoplasma hominis (29,47,68,94,95,98,160). Animal experiments with grivet monkeys suggest that M hominis gives rise to parametritis rather than salpingitis (103,106,107). Whether or not this is also the case in humans has not been established.

Several other organisms, such as species of Bacteroides, Clostridium, Peptococcus, Peptostreptococcus, Haemophilus influenzae, Campylobacter fetus and group A streptococci have been associated with some cases of PID (for reviews see 29,47,68,95,160). In our hospital catchment region, these organisms play a minor role as aetiological agents in AS (95,185,187).

Species of Actinomyces, generally A israelii, when demonstrated in AS patients, seem in the majority of cases to be associated with use of IUDs (29,47,95).

Although several microbial species have been associated with PID, a large proportion of all PID cases have remained "unexplained". That Chlamydia trachomatis was yet another micro-organism implicated in PID was first suggested in 1964 (25). Since then several studies have confirmed such a connection (24, 31,64,93). The first isolations of C trachomatis from the fallopian tubes in women with AS were made in Sweden during the mid-1970's (28,185).

Chlamydia

Within the genus Chlamydia, there are two species, C trachomatis and C psittaci. C trachomatis has 15 biovars; A, B, Ba, C, D, E, F, G, H, I, J, K and L1, L2 and L3 (114,138,148). The chlamydiae are prokaryotic organisms, which are obligate intracellular parasites. They occur extracellularly as infectious, nonreplicating elementary bodies (EB). The EB's become phagocytosed by the host cells into intracytoplasmatic inclusions inside which the chlamydiae are reorganized to initial bodies (IB), which are the reproductive forms (114,148,149,168). After a series of binary fissions the IB's condense to form new EB's. The EB's are then released from the host cell which bursts.

Diagnosis of chlamydial infections

The main method for the diagnosis of infections by C trachomatis has up to now relied on the isolation of the agent. The demonstration of the organism by cytological methods directly in clinical material and serology has so far been of less importance.

The first isolation of C trachomatis was reported in 1957, when the organism was isolated in embryonated hens' eggs (167). In 1965 a cell culture technique was utilized to recover chlamydiae (45). Since then several such techniques have been described (23,138). In the present studies, cycloheximide-treated McCoy cells were used for the isolation of chlamydiae (139). The main advantage with the cycloheximide method is that treatment of the cell cultures can be made in conjunction with the infection of the cells (138). In comparative study experiments for the isolation of C trachomatis, cycloheximide treatment gave a significantly higher inclusion count, than use of pre-treatment techniques (34).

Giemsa staining and immunofluorescent (IF) procedures have been used for the demonstration of chlamydial inclusions directly in clinical samples. However, these methods have been found to be relatively insensitive when applied to the diagnosis of genital infections (147). The use of monoclonal antibodies in IF tests to detect both cytoplasmatic inclusions and elementary bodies has recently been reported to give results comparable to those obtained by cultural methods (166).

Of the different serological tests used to detect antibodies to C trachomatis, the MIF test has so far been the most widely used (114,174,181). The discussion to follow on serology is limited to results obtained with the MIF test (174,175,179-181). In studies II-V, pools of antigens consisting of biovars A-C, D-K (except J) and L1-3 were used (175):

Recently, enzyme-linked immunosorbent assays (ELISA) have been used to detect serum antibodies to C trachomatis (83,155). ELISA appears to have the same sensitivity as MIF tests (83,155).

Chlamydia trachomatis in human disease

"Because chlamydiae are obligatory intracellular parasites, they are (at a cellular level at least) true pathogens, and do not simply persist on mucous membranes or cell surfaces" (Schachter 1978 /146/).

In a variety of human diseases, C trachomatis have been incriminated. The aetiological role of C trachomatis in some of these diseases, seems to be unequivocal, while in others, the evidence for an aetiological role is less

strong. A tentative grading of the evidence supporting the aetiological role of C trachomatis in a number of diseases is shown in Table 1.

Isolation of C trachomatis from the female lower genital tract

In women attending Sexually Transmitted Disease (STD) clinics, the isolation rate of C trachomatis has been found to vary between 8 and 38 % (Table 2). In female contacts with chlamydia culture-positive males, up to 68 % of the women proved to be culture-positive for this organism (Table 3). The corresponding figure for contacts with men with chlamydia-negative nongonococcal urethritis has, at the most, been 18 % (Table 3). In women harbouring gonococci in the lower genital tract, C trachomatis has been recovered in 27 to 63 % of the cases (Table 4).

Generally, the isolation rate of C trachomatis has been lower in non-STD-clinic patients. In pregnant (or puerperal) women, chlamydiae have been recovered from 2 to 22 % (Table 5). From gynecological out-patients an isolation rate of 1 to 15 % of this organism, has been reported (Table 6). In screenings, 1 to 7 % of the women have been found to harbour chlamydiae (Table 6). In the majority of the reported studies, C trachomatis was isolated more frequently than N gonorrhoeae. In screenings of women without symptoms, the ratio between chlamydial and gonococcal infections was found to be approximately 10:1. In the UK and the USA, genital C trachomatis infections now outnumber N gonorrhoeae infections (15,54,67). The results from several studies suggest that this is also true in the Scandinavian countries (96).

The prevalence of genital chlamydial infection is age dependent. The younger the woman, the higher the isolation rate of genital C trachomatis (115,121, 128,141). In women consulting STD clinics and gynecological outpatients clinics, 64 to 79 % of those harbouring chlamydiae in the lower genital tract were below the age of 25 years (7,64,79,128,164). Similar findings have been reported from studies of puerperal or pregnant women (39,100,129) and in women seeking abortion (100,182).

This age dependency of the incidence of C trachomatis infection might be due to immunity factors (121). Furthermore the frequent change of sexual partners is more common among young people, and consequently the younger women are at higher risk of acquiring an STD (54,66,121). An additional hypothesis put forth is that cervical ectopy could provide a larger area of accessible columnar epithelium for chlamydial infection (146). Ectopy is defined as an extension of the columnar epithelium in the cervical canal to the area outside the external cervical orifice normally covered with squamous epithelium. Because such a cer-

Table 1. Assessment of evidence, that Chlamydia trachomatis, biovars A-K, plays an aetiological role in various diseases

Disease	Evidence supporting that chlamydia may be a cause		
	<u>Strong</u>	<u>Fairly strong</u>	<u>Weak</u>
<u>Children</u>			
Ophthalmia neonatorum	+		
Rhinitis			+
Nasopharyngitis			+
Acute otitis media			+
Pneumonia	+		
Vulvitis		+	
<u>Adults</u>			
Trachoma	+		
Conjunctivitis	+		
Keratitis punctata	+		
Mb Reiter		+	
Meningo-encephalitis			+
<u>Males</u>			
Urethritis	+		
Epididymitis	+		
Prostatitis			+
Proctitis		+	
<u>Females</u>			
Urethritis	+		
Bartholinitis			+
Cervicitis	+		
Cervical dysplasia			+
Endometritis		+	
Salpingitis	+		
Perihepatitis	+		

Adapted from ref. 67,96,114,146,168

Table 2. Isolation rate of Chlamydia trachomatis from cervical and/or urethral specimens in women attending STD clinics

Country	No. in ref. list	Year	No. isolation positive/ No. examined	%	Comment
UK	116	1974	45/ 247	18	First time visitors
UK	64	1974	86/ 279	31	Unselected
UK	65	1974	38/ 190	20	With complaints
UK	86	1975	76/ 597	13	Fresh complaints
UK	109	1976	60/ 300	20	First time visitors
UK	137	1977	269/1136	24	Unselected
Sweden	75	1977	49/ 130	38	Symptoms of genital in- fection or named as contacts
UK	192	1977	53/ 200	27	"Promiscuous"
UK	115	1978	58/ 284	20	Unselected
UK	176	1978	35/ 272	13	Unselected
Sweden	128	1979	189/ 755	25	First time visitors
USA	150	1979	36/ 180	20	Unselected
UK	136	1980	154/ 796	19	First time visitors
Sweden	76	1980	158/ 585	27	First time visitors
UK	165	1980	70/ 202	35	NGU contacts. Women with gonorrhoea excluded
Sweden	170	1980	207/1039	20	Unselected
Canada	11	1981	113/ 452	25	Unselected
USA	14	1982	26/ 69	38	Women with known or suspected gonorrhoea
UK	113	1982	136/ 536	25	First time visitors
Spain	26	1982	8/ 107	8	Unselected
UK	43	1982	20/ 53	38	Pregnant

STD = Sexually Transmitted Disease

NGU = Non-Gonococcal Urethritis

Table 3. Isolation of Chlamydia trachomatis from the cervix and/or the urethra in contacts of men with nongonococcal urethritis

Country	No in ref. list	Year	Culture-positive/No tested			
			Contact chlamydia- positive	%	Contact chlamydia- negative	%
UK	117	1972	12/ 18	67	1/23	4
USA	69	1975	15/ 22	68	2/24	8
USA	55	1976	15/ 24	63	1/21	5
UK	3	1977	14/ 31	45	14/77	18
Finland	122	1978	25/ 39	64	3/36	8
Finland	169	1978	22/ 63	63	2/22	9
Eire	87	1978	9/ 21	43	2/32	6
Canada	40	1979	7/ 11	64	1/10	10
Sweden	170	1980	67/103	65	Not stated	-

Table 4. Isolation of Chlamydia trachomatis from women harbouring Neisseria gonorrhoeae

Country	No. in ref. list	Year	No. chlamydia-positive/No. tested	%
UK	116	1974	9/ 28	32
UK	64	1974	36/ 57	63
UK	86	1975	14/ 32	44
UK	109	1976	18/ 66	27
Sweden	75	1977	25/ 52	48
UK	137	1977	75/205	37
UK	192	1977	13/ 32	41
UK	115	1978	15/ 48	31
Sweden	128	1979	NS/755	40
Sweden	76	1980	62/160	39
Canada	11	1981	18/ 38	47
USA	14	1982	23/ 56	41

NS = Not Stated

Table 5. Isolation of *Chlamydia trachomatis* from the cervix and/or the urethra in pregnant women

Country	No. in ref. list	Year	No. positive/No. examined	%	Comment
USA	4	1977	18/ 142	13	Unselected
USA	151	1979	36/ 900	4	Up to four months pregnant
USA	39	1979	30/ 340	9	Low risk pregnancies
USA	52	1979	6/ 322	2	Second trimester
USA	53	1980	23/ 107 44/ 465	22 10	Different clinics. Third trimester Different clinics. Third trimester
Sweden	100	1980	14/ 231 23/ 273	6 8	Seeking abortion Puerperal
USA	178	1980	45/ 486	9	Third trimester
Sweden	129	1981	32/1328	2	Puerperal
USA	58	1981	240/1327	18	Black, urban women
USA	57	1982	78/1125	7	First and third trimester
Norway	132	1982	30/ 218	14	Seeking abortion
Denmark/ Sweden	105	1982	23/ 432 25/ 511	5 5	Seeking abortion Seeking abortion
Denmark	182	1982	33/ 333	10	Seeking abortion
USA	173	1982	71/ 433	16	First and third trimester

Table 6. Isolation of *Chlamydia trachomatis* from the cervix and/or the urethra of women attending other clinics than STD clinics

Country	No. in ref. list	Year	No. positive/ No. examined	%	Clinic	Comment
Sweden	141	1978	32/ 215	15	Gynecologic	Symptoms of infection. Women with bleeding excluded
Finland	123	1978	13/ 144	9	Gynecologic	Unselected
Sweden	164	1981	298/3490	9	Gynecologic	Symptoms of infection. Women with AS excluded
Italy	16	1981	1/ 106	1	Gynecologic	Women without signs of infection
			9/ 160	6	Gynecologic	Women with cervical ectopia
UK	64	1974	2/ 63	3	Family Planning Association	Contraceptive advice
USA	152	1975	23/ 665	3	Screening Acute care Women's clinic	Asymptomatic Symptomatic
UK	192	1977	2/ 200	1	Hospital staff	Contraceptive advice
USA	88	1979	9/ 185	5	Students	
Sweden	128	1979	10/ 201	5	Health control	
USA	90	1981	20/ 439	5	Students	Cystourethritis symptoms Sexually active
Canada	11	1981	9/ 123	7	Students	
Canada	13	1981	31/ 119	26	Urologic	
USA	145	1981	22/ 100	22	Adolescent	Symptomatic Asymptomatic
Kenya	111	1982	11/ 210	5	Various clinics	
Gambia	85	1982	6/ 33	18	Medical research	
USA	118	1982	24/ 253 9/ 130	9 7	Gynecologic	

STD = Sexually Transmitted Disease

vical columnar epithelial ectopy is more common in young women (19,44), and chlamydia probably infects only columnar epithelium, this might be a contributing factor to the age differences observed in the prevalence of cervical chlamydial infection.

AIMS OF THE INVESTIGATION

- To determine the possible role of Chlamydia trachomatis as an aetiological agent in acute salpingitis,
- to describe the histological changes in the fallopian tubes in chlamydia tubal culture-positive salpingitis cases,
- to assess the proportion of salpingitis cases caused by C trachomatis,
- to depict the clinical and laboratory findings characteristic of chlamydial salpingitis,
- to evaluate the prognosis after chlamydial salpingitis with special emphasis on fertility.

MATERIAL AND METHODS

Patient material

The patient material comprised women with laparoscopically verified AS, treated at the department of Obstetrics and Gynecology, University Hospital, Lund, Sweden, during the years 1975 to 1981.

Diagnostic procedures

Provisional clinical diagnosis The minimum criteria for the provisional clinical diagnosis of AS in studies I-VI were as follows: the patient complained of bilateral, low abdominal pain and had, on examination, adnexal tenderness. In addition, microscopy of a wet smear of the vaginal contents indicated a genital infection, i.e. the number of leukocytes in the vaginal secretion outnumbered all other cellular elements. In women with vaginal bleeding in whom the wet smear was impossible to analyse at least two of the following signs and symptoms had to be present for the provisional diagnosis of AS: history of an abnormal vaginal discharge, menstrual irregularities, vomiting, proctitis, urgency and/or pain on voiding, fever and erythrocyte sedimentation rate (ESR) ≥ 15 mm/h (73).

Laparoscopy was performed with the patient under general anaesthesia, muscle relaxation and with controlled breathing. The pneumoperitoneum was established by insufflation of three to four liters of CO₂. The optical instrument was introduced through a transverse incision made at the lower border of the umbilicus. Smaller incisions were made in the hypogastric fossae. Through these incisions manipulation and/or specimen-collection probes were introduced into the pelvic cavity.

Minimum criteria of a laparoscopic diagnosis of acute salpingitis, as used in these studies, were reddening (hyperaemia) and swelling (oedema) of the tubes. The most important criterium was, that purulent or seropurulent exudate covered the tubal surfaces or oozed through the abdominal orifices when patent. With manipulation probes such oozing can be produced. Occasionally the other two criteria were not regarded as necessary if such oozing was produced as this indicates endosalpingitis (78).

Grading of the inflammatory changes of the tubes, as seen during laparoscopy, were 1) mild: minimum criteria. The tubes freely movable and with the abdo-

minimal orifices patent. 2) Moderately severe: as (1) but more marked changes, the tubes not freely movable, patency of the tubes uncertain. 3) Severe: pelveoperitonitis and/or abscess formation.

The acquisition of specimens for the isolation of C trachomatis and N gonorrhoeae is described in study I. In principle, these methods have not changed, except that now (since 1980) a specially designed, 30 cm long, cotton-tipped aluminum swab is used for the collection of intra-abdominal specimens (ENT^R, Medical Wire and Equipment Co. Ltd, Corsham Wiltshire, England). The specimens for the isolation of C trachomatis were transported in sucrose-phosphate buffer (2-SP) (46), and those for N gonorrhoeae as well as M hominis in modified Stuart's medium (50).

Serology The MIF test was used for the detection of antibodies to C trachomatis. Indirect haemagglutination (IHA) tests were used for the detection of antibodies to N gonorrhoeae and M hominis (81,135).

Treatment Immediately after laparoscopy, antibiotic treatment was started. The treatment given was either 0.5 g ampicillin four times daily (during 1975 and part of 1976), or doxycycline 200 mg the first day, followed by 100 mg daily, (1976 - autumn 1979). Since the autumn of 1979, patients have been given 200 mg doxycycline daily. The first dose of the antibiotic was given intravenously, the following doses were taken orally. This therapy was given for at least 10 days. During this time, the patients were treated in hospital with bed rest.

Contact-tracing control and follow-up Partners of patients infected with chlamydiae and/or gonococci were requested to consult the hospital's STD clinic.

The AS patients were checked three or four weeks after the end of treatment at the gynecological clinic's out-patient department. On this occasion a bimanual pelvic examination was performed, a wet smear examined and the ESR (mm/h) determined. In women who had had positive cultures for chlamydiae and/or gonococci on admission cervical specimens were obtained for bacterial isolation studies.

In 1982, a follow-up of the AS patients, treated during the period 1975 to 1979, was performed. Only cases where specimens for the isolation of C trachomatis and N gonorrhoeae had been obtained, and where these specimens had not been spoiled, were included in this follow-up. Questionnaires were sent to the women regarding their fertility/infertility after the AS episode(s). The information obtained in the questionnaires was, where possible, cross-checked

in hospital records.

EVIDENCE THAT CHLAMYDIA TRACHOMATIS CAN CAUSE SALPINGITIS

General aspects

AS is in general the result of an infection starting in the lower genital tract. However, the fact that an organism cannot be recovered from the cervix in AS patients does not contradict an aetiological role of that agent in a given case of AS. Many series illustrate a discrepancy between the results of isolation attempts from the lower genital tract and findings from the endometrium and the fallopian tubes. Isolation attempts from the lower genital tract should, however, always be undertaken, e.g. for epidemiological reasons.

Isolation of a micro-organism from the fallopian tubes in patients with AS provides strong evidence for the aetiological role of the organism in this disease.

The demonstration of a significant antibody response (defined as seroconversion or at least a four-fold change in titres of antibodies) to the imputed agent is further supporting evidence.

Koch's postulates state that one proof of a given micro-organism causing an infection is: 1) the micro-organism must be regularly isolated from cases of the illness; 2) it must be isolated in pure culture; 3) the organism must (when inoculated into a susceptible animal) reproduce the disease; 4) from such animals, the organism must again be isolated. However, while these postulates were adequate to prove the aetiology of some bacterial disease, they had to be modified to be applicable to other infectious diseases, due to e.g. species' specificity of certain agents and that in some instances synergistic infections are required for clinical disease to occur.

Isolation of Chlamydia trachomatis from the lower genital tract in women with acute salpingitis (I,III)

In Scandinavian studies on PID, C trachomatis has been isolated from the lower genital tract in 22 to 56 % of the patients studied (Table 7). The isolation rates of C trachomatis in PID from other parts of the world have been found to vary between 5 and 51 % (Table 7).

The corresponding figures for N gonorrhoeae and M hominis are shown in Table 8. Since 1976, the incidence of gonorrhoea in Scandinavian PID patients has varied between 5 and 32 % (Table 8).

In studies of PID patients outside Scandinavia, the corresponding percent-

Table 7. Isolation of *Chlamydia trachomatis* from cervical and/or urethral specimens of women with pelvic inflammatory disease

Country	No. in ref. list	Year	No. chlamydia culture-positive/ No. examined*	%
USA	32	1975	20/100	20
Sweden**	28	1976	6/ 22	27
Sweden**	I	1977	19/ 53	36
Finland	120	1980	69/228	30
France**	60	1980	6/ 16	38
Sweden**	142	1980	52/156	33
USA**	162	1980	2/ 39	5
USA**	172	1980	3/ 30	10
Sweden**	III	1981	23/ 60	38
Canada	10	1981	22/ 43	51
Denmark	108	1981	37/166	22
Norway**	42	1982	26/ 56	46
UK	2	1982	4/ 78	5
Sweden	119	1982	52/111	47
Norway	155	1982	19/ 34	56
Denmark	9	1982	15/ 56	27

* Non-valid specimens excluded

** Laparoscopically verified acute salpingitis

Table 8. Isolation of Neisseria gonorrhoeae and Mycoplasma hominis from the cervix and/or the urethra of women with pelvic inflammatory disease. Same studies as in Table 7

No. in ref. list	Year	<u>Neisseria gonorrhoeae</u>		<u>Mycoplasma hominis</u>	
		No. positive/No. examined	%	No. positive/No. examined	%
32	1975	90/204	44	146/204	72
28	1976	7/ 22	32	9/ 22	41
I	1977	11/ 53	21	ND	
120	1980	60/228	26	ND	
60	1980	2/ 17	12	NS	
142	1980	39/206	19	ND	
162	1980	19/ 39	49	28/ 39	72
172	1980	24/ 30	80	10/ 30	33
III	1981	4/ 60	7	ND	
10	1981	15/ 43	35	ND	
108	1981	9/166	5	91/166	55
42	1982	5/ 65	8	ND	
2	1982	14/ 78	18	ND	
119	1982	41/209	20	ND	
155	1982	NS		NS	
9	1982	9/ 56	16	ND	

ND = Not Done; NS = Not Stated

Table 9. Isolation of Chlamydia trachomatis from the cervix and/or the urethra from the fallopian tubes in women with acute salpingitis

Country	No. in ref. list	Year	Isolation of C trachomatis*	
			Cervix and/or urethra No. positive/No. examined	Fallopian tubes No. positive/No. examined
Sweden	28	1976	6/22	2/22
Sweden	I	1977	19/53	6/20
USA	162	1980	2/39	0/35
USA	172	1980	3/30	3/30
France	60	1980	6/16	4/17
Norway	42	1982	26/56	5/42

* Non-valid specimens excluded

ages are 12 to 80.

It thus seems clear, that in the Scandinavian countries C trachomatis is recovered more frequently than N gonorrhoeae from the lower genital tract in women with PID. The difference in the isolation frequencies between the studies cited, could be due to: a) variations in the prevalence of the organisms in various populations, b) patient selection bias caused by differences in health care systems, c) varying criteria for the diagnosis of PID, and d) different quality of the methods used for sample collection and handling of specimens.

In four of the studies cited in Table 8, specimens for the isolation of M hominis were also obtained. The isolation frequencies from the lower genital tract for this organism varied between 33 and 72 %.

Double infections with C trachomatis and N gonorrhoeae in the lower genital tract in women with AS have been reported in 2 to 23 % of the cases studied (9,10,32,108,119,120).

Only one study has reported the results of simultaneous isolation attempts for C trachomatis and M hominis from the lower genital tract in women with PID (108). In this study, 21 (13 %) of the 166 patients harboured both organisms in the cervix (108).

Thus it is clear, that STD agents often occur concomitantly in the lower genital tract in women with PID. As mentioned above caution must be exercised when interpreting isolation results from the lower genital tract. In specimens obtained from the fallopian tubes, more than one STD agent is generally not recovered.

Isolation of Chlamydia trachomatis from the fallopian tubes in women with acute salpingitis (I,V)

In 1976, the first reports were published on the successful recovery of C trachomatis from the fallopian tubes in women with AS (28,51,185). Up to, and including, 1982, at least six series of isolation attempts of chlamydiae from the fallopian tubes and/or pouch of Douglas have been published (Table 9). In all but one of these studies (172), the specimens were obtained exclusively during laparoscopy.

In study I, C trachomatis was isolated from the fallopian tubes in six of 20 tubal specimens. Of seven women harbouring chlamydiae in the cervix and from whom tubal specimens were also obtained, the organism was recovered from the fallopian tubes in all but one (I). In five of the six studies cited in Table 9, C trachomatis could be recovered from the tubes. In the only study

which failed to isolate the organism, culture attempts were made on tubal exudate (162), while most of the other investigators used swabs for the collecting of specimens (1,42,60,172).

In study I, chlamydiae could not be recovered in any of the 29 specimens obtained from the cul-de-sac. In an extension of this series, C trachomatis was recovered in only one of 107 specimens obtained from the pouch of Douglas in women with AS (138). Only occasionally have isolation attempts for chlamydiae from the cul-de-sac been successful (32,59). It was found that sampling from the tubes gives a higher yield (1).

Neither chlamydiae, nor gonococci could be isolated from the lower genital tract in 12 controls (1). These controls were without signs or symptoms of a genital infection, but subjected to laparoscopy for other reasons, such as chronic abdominal pain or laparoscopic sterilization. Tubal specimens obtained from five of these controls were all culture-negative for chlamydiae (1).

One of the women in study I, in whom chlamydiae were isolated from the tubes, had no reddening or swelling of the tubal walls. With the aid of the manipulation probes, it was, however, possible to elicit seropurulent exudate from the abdominal orifices of the tubes. One case has been published of the recovery of chlamydiae from the fallopian tubes in a woman with no apparent signs of infection during laparoscopy (61). This woman had an extra-uterine pregnancy six months later, the removed tube showed histological evidence of a past mucosal inflammation (61). Thus it seems likely that an early, mild chlamydial tubal infection can occur as an endosalpingitis with no or few visible signs of infection on the external surfaces of the tubes.

Gonococcal endosalpingitis with laparoscopically normal tubes has been described (78). In this study, as well as in the study of Henry-Suchet et al. (61) it was not stated if attempts were made to express seropurulent or purulent exudate from the tubes, nor if such an exudate covered the tubal surfaces (see Material and Methods, page 18).

In study V, 10 women are described, who were all culture-positive for C trachomatis from the fallopian tubes. In seven of these patients, chlamydiae could also be recovered from the cervix, in one case the culture was spoiled (V). In two other cases the cultures obtained from the lower genital tract were negative for C trachomatis (V). Failure to isolate C trachomatis from the cervix, although the organism could be obtained from the tubes, has been reported by others (28,42).

One of the women in study V, who was culture-negative for C trachomatis from the cervix and the urethra on admission, had harboured the organism in

the cervix three weeks earlier. This woman had no antibodies in cervical secretions on admission, although such antibodies were detected nine days and one month after the admission (unpublished). Whether local antibodies might interfere with the recovery of C trachomatis is not known. Poor sampling or specimen handling might be other reasons for a discrepancy between culture results from the cervix and the fallopian tubes. Specimens from both the urethra and the cervix were obtained approximately 15 hours apart before antibiotic treatment was started in one of the two patients who were culture-negative from the lower genital tract (V) (unpublished). In one of the patients in study V, C trachomatis could be recovered from the endometrium and the fallopian tubes, but not from the cervix.

Subhuman primates, experimentally infected with C trachomatis directly in the tubes, have shed the organism from the cervix, even when chlamydiae could not be reisolated from the tubes (125,140).

Antibodies to Chlamydia trachomatis in acute salpingitis (III,V)

In women with PID, a significant change in titres of antibodies to C trachomatis could be due to: a) a lower genital tract infection due to the organism; b) chlamydial endometritis and c) salpingitis caused by the organism. Antibodies formed against other organisms and cross-reacting with C trachomatis could be another explanation. In the MIF test used in our studies, such cross-reacting antibodies have so far not been reported. Findings of significant changes in titres of chlamydial antibodies in women with AS strengthen the probability that C trachomatis is the etiological agent of the salpingitis in the patient under study.

It is not always possible to demonstrate a significant change in titres of chlamydial antibodies in women with PID, even in patients culture-positive from the lower genital tract or the fallopian tubes (V). This might be explained by improperly spaced drawings of sera or a preceding chlamydial infection of the cervix and/or the endometrium which might have provoked an antibody response before the AS patient consulted a doctor (97,144,147,174). The insidious or chronic nature of many chlamydial infections are well documented (for reviews see ref. 144,147,174,181).

Detection of serum IgM antibodies to C trachomatis is by most workers regarded as proof of a current chlamydial infection, as these antibodies are relatively short-lived (12,144,146,168,174,181). However, in occasional instances, such antibodies may persist for several months (181). IgM antibodies do not appear regularly, even in a primary chlamydial infection (174,181), and

are generally not recalled in a reinfection of the original or different biotype (174,181). Thus the value of IgM antibodies in the diagnosis of chlamydial infections is limited.

In studies of PID patients, a significant change in titres of antibodies (IgM and/or IgG) to C trachomatis has been reported in 19 to 46 % of the women (Table 10). In women with PID, culture-positive for chlamydiae from the lower genital tract, such a change in titres has been found in 31 to 54 % of the cases (Table 11).

The results obtained in study III, using the MIF test, are in agreement with other similar studies of women with AS (Tables 10,11).

As in other studies of AS, a high percentage of culture-negative women had a significant change in the titre of chlamydial antibodies (III). Possible explanations for this have already been discussed (page 25). Regarding our salpingitis patients, it could not be excluded, that some of the patients had been treated with antibiotics before admission, which would explain why they were culture-negative from the lower genital tract.

In study III we found that the initial titres of chlamydial IgG antibodies (subdivided into three groups: ≤ 64 , 128-256 and ≥ 512), in women who had or developed such antibodies during the disease, was correlated to the severity of the inflammatory condition of the tubes (mild, moderately severe and severe, page 18) ($p < 0.005$), and also with the duration of pelvic pain before admittance (1-3; 4-6; 7-9; 10 or more days) ($p < 0.009$). If the duration of pelvic pain can be taken as indicative of the duration of the tubal infection, then such longstanding infections generally give rise to more severe inflammatory reactions of the tubes as well as to high titres of the antibodies to C trachomatis.

Evidence of a current chlamydial infection (defined as positive culture or a significant change in titres of antibodies or both) was found in 35 (58 %) of the 60 patients in study III. The corresponding figure for N gonorrhoeae was 5 (8 %). Significant change in the titres of IHA antibodies to M hominis, occurred in 7 (12 %) of the patients, four of whom also had a significant titre change to C trachomatis (III). These figures indicate, that C trachomatis is currently the most important aetiological agent of AS in our hospital catchment region, while N gonorrhoeae nowadays plays a minor role in this region as a cause of AS.

In study V, two of the women with C trachomatis isolated from the fallopian tubes, did not show a significant change of titres of chlamydial antibodies. One of these two women had a two-fold change in titres of such antibodies. How-

Table 10. Significant change in titres of antibodies to Chlamydia trachomatis in women with acute pelvic inflammatory disease

Country	No. in ref. list	Year	No. with significant change/No. tested	%
USA	32	1975	15/ 74	20
USA*	162	1980	5/ 22	23
Finland	120	1980	32/167	19
Sweden*	142	1980	28/ 80	35
Sweden*	III	1981	22/ 60	37
Denmark	108	1981	34/166	20
Sweden	119	1982	25/ 72	35
Norway*	42	1982	24/ 52	46

* Laparoscopically verified

Table 11. Isolation of Chlamydia trachomatis from the lower genital tract related to significant change in titres of antibodies to the organism in women with pelvic inflammatory disease

Country	No. in ref. list	Year	Number (%) with significant change in titres			
			<u>Chlamydia trachomatis</u>			
			Isolated		Not isolated	
			n/N*	%	n/N*	%
USA	32	1975	5/19	26	10/ 55	18
Finland	120	1980	17/54	31	51/113	13
Sweden**	III	1981	10/23	43	12/ 37	32
Denmark	108	1981	20/37	54	14/129	11
Norway**	42	1982	13/25	52	7/ 27	26

* n = Number of women with significant change in titre

N = Number of women studied

** Laparoscopically verified

ever, in this patient the time between the collection of sera was rather short, i.e. five days (V). The other woman had a stationary titre. The inflammatory changes of the tubes in these two patients were moderately severe and mild, respectively (V). Both these women harboured N gonorrhoeae in the cervix and both had IHA antibodies to this organism (V). The patient with a stationary titre of chlamydial antibodies showed a significant decrease in gonococcal IHA antibodies, although this organism could not be isolated from the tubes (V).

Antibodies to C trachomatis, N gonorrhoeae and M hominis were detected in 80 %, 18 % and 40 % of the cases, respectively (III). The corresponding figures for 50 pregnant controls were 8 %, 6 % and 8 % (III).

Thus, the patients with AS had abundant serological evidence of a past (or current) infection from STD's (III).

Animal experiments

Animal species have been used in the study of chlamydial diseases (for review see ref. 8). In the study of chlamydial salpingitis caused by biovars D-K, rabbits and nonhuman primates have been used (104,107,125,126,140).

Rabbits, inoculated directly in the oviducts, developed an acute self-limiting salpingitis (126).

In female grivet monkeys, human genital strains of C trachomatis give rise to an acute self-limiting salpingitis (104,107,140). In monkeys inoculated directly into the tubes, chlamydiae could be recovered from the cervix, but not from the tubes (140). The spread of chlamydiae in the genital tract of the grivet monkeys is probably canalicular (104,140).

Microscopically, there was infiltration of the subepithelial tissues of the tubes, involving the epithelium before macroscopic signs of inflammation was apparent (140). In some of the monkeys, tubal occlusion developed (104,107). The serological response was an IgM to IgG conversion, with the disappearance of detectable IgM antibodies within five to eight weeks (104,107,140).

Female pig-tailed macaques also developed an acute self-limiting salpingitis, when inoculated with chlamydiae (125). The histopathological changes of the tubes resembled those found in the grivet monkeys (125,140). In these monkeys, the IgM antibodies persisted during the observation period, i.e. for at least 12 weeks (125).

Koch's postulates were fulfilled in these monkey experiments (104,107,125,140).

In both these species of sub-human primates, there were no clinical signs of infection (104,107,125,140). A modest increase in the ESR mm/h were noted

in the grivet monkeys (140).

The histopathological findings of the tubes in these sub-human primates, closely resembled our findings in women with chlamydial salpingitis (II).

PATHOPHYSIOLOGY OF CHLAMYDIAL SALPINGITIS

Mode of spread

There is general agreement that in gonococcal salpingitis, the spread of the infection from the lower genital tract to the tubes is canalicular via the endometrium (56,66,133,159). This mode of spread is probable also in chlamydial salpingitis. This assumption is supported by animal experiments (104,107). The finding of chlamydial endometritis in cases of salpingitis might also serve as indirect evidence of this mode of spread (48,70,101,161, 193,194). Cornual resection prevents recurrence of PID which also argues for a canalicular spread (35).

Risk factors

The prevalence of chlamydial cervicitis is age dependent (see page 11). In study IV, it was observed that women with C-AS were significantly younger than women with NCNG-AS, but that there was no age difference between C-AS and G-AS patients.

It could be postulated that promiscuity is a risk factor. "PID among sexually inactive women virtually never occurs except in the presence of an IUD or recent genital tract instrumentation" (Eschenbach 1980 /30/).

In women with a genital chlamydial infection, a correlation has been reported by the majority of investigators to the use of oral contraceptives (OC) (7, 64,121,141,164,165), others have not found such a correlation (109,192). The reason for an association between OC use and chlamydial recovery is at present unclear. However, cervical ectopy has been found to be correlated with chlamydial recovery (86,115,152,165), age (19,44) and OC usage (44,86,143) (see page 11).

Most studies have not observed any association between the use of IUD's and recovery of chlamydiae (115,121,141). In one study women over the age of 19 showed no correlation between the use of copper-medicated IUD's and the rate of recovery of C trachomatis from the cervix (164). In this context, it is of interest that CuCl_2 caused a decrease in the inclusion counts of experimentally infected cell cultures, using a standardized inoculation dose of C trachomatis (99).

On the other hand, the risk of an ascending cervical infection seems generally to be less in women using OC's, compared with those women using IUD's, other, or no contraceptives (27,29,153). This has been explained by the changed properties of the cervical mucus by gestagenic hormones, making it more difficult for organisms to enter the uterine cavity (29,189). It has also been suggested that the decreased myometrial activity observed in OC users might lessen the risk of an upward propulsion of bacteria (188). With respect to contraceptive usage and AS, women with C-AS do not seem to differ from women with AS caused by other agents (42). In study IV, 41 % of the C-AS patients used IUD's, while 24 % used OC. In women with G-AS and NCNG-AS the corresponding figures were 58 and 34 % for IUD use, and 16 and 17 % for use of OC, respectively. Similar proportions of IUD and OC usage in C-AS patients have been reported by others (42).

In study IV, factors predisposing to AS, such as insertion of an IUD, curettage or hysterosalpingography (within four weeks of admission), were reported significantly more often by C-AS than by G-AS or NCNG-AS women. Others have reported such procedures proceed the acute episode more frequently in studies comparing women with "gonococcal" PID with women with "nongonococcal" PID (73,185). Women culture-positive for chlamydiae and applying for abortion, have a higher risk of developing PID post abortum than chlamydia culture-negative women (105,132,182).

These findings suggest that chlamydial infection of the cervix is often asymptomatic and might be overlooked in clinical examinations. Further evidence of many cases of chlamydial cervicitis being asymptomatic can be found in Tables 5 and 6. These observations suggest that chlamydial infection should be excluded before procedures are performed which implies the entering of the endometrial cavity.

Gross lesions

The tubes in chlamydia culture-positive cases of salpingitis have shown changes ranging from seropurulent or purulent exudate which could be expressed from the abdominal orificis of the tubes, but with no other apparent signs of tubal infection (see page 24) via reddened and swollen adhered tubes up to pelvic peritonitis with abscess formation (see Material and Methods, page 18).

Microscopic appearance

The histopathological findings in human chlamydial salpingitis have, up to date, been described in two cases (II). In one of the cases, chlamydial inclu-

sions were found in Giemsa-stained specimens, obtained from the tubes, and examined by dark-field illumination. Specimens from the tubes in this case were culture-negative for N gonorrhoeae and M hominis. In the other patient, C trachomatis was isolated directly from the fallopian tubes. N gonorrhoeae, anaerobes, facultative anaerobes, aerobes and mycoplasmas could not be recovered from tubal specimens in this patient.

Histological examination of the tubes in these two patients, showed marked inflammatory response through all layers of the tubes. The lumina were filled with an exudate containing lymphocytes and desquamated epithelial cells in Case 1, and polymorphonuclear leukocytes in Case 2. The epithelium of the tubes was patchily injured, oedematous and infiltrated with inflammatory cells. Also the muscle layers and the subserosa were infiltrated with lymphocytes and polymorphonuclear leukocytes.

The light-microscopy findings of the tubes in these two patients, were the same as those described in standard text books of gonococcal salpingitis (110).

Similar histopathological findings were found in the tubes of subhuman primates, experimentally infected with C trachomatis and which developed signs of salpingitis (104,107,125,140). Exudates containing lymphocytes and desquamated epithelial cells as well as lymphocytes and leukocytes infiltrating the tubal walls were found in these monkeys (104,107,125,140). Adhesions between the mucosal folds have also been noted in such animals; similar to the description of human gonococcal salpingitis (22).

A specimen from the fallopian tube of Case 2, was also studied by transmission electron microscopy. No cellular inclusions consistent with those caused by chlamydia were seen in the few sections studied. The cilia of the ciliated cells did not show any structural changes. The nuclei of the ciliated cells showed degenerative alterations with distorted morphology. In non-ciliated cells, an increased number of lysosomes was found, suggesting an increased secretory activity.

CLINICAL MANIFESTATIONS AND LABORATORY FINDINGS IN CHLAMYDIAL SALPINGITIS

Studies of women with PID, comparing patients with N gonorrhoeae isolated from the lower genital tract with patients culture-negative for this organism, have reported that women with "gonococcal" PID: 1) are younger (172,185); 2) more often have febrile illness (73,185); 3) have onset of pain with menses (33,91); 4) have increased vaginal discharge (30,32,73) and respond to antibiotic therapy sooner (20,91,172) than women with "nongonococcal" PID.

Because in our region, the majority of chlamydia infected salpingitis cases do not harbour N gonorrhoeae in the lower genital tract, it may be possible to evaluate differences with respect to the points mentioned above between patients with C-AS, G-AS and NCNG-AS.

In study IV, patients with AS were subdivided with regard to the results of chlamydial and gonococcal cultures and tests for antibodies to C trachomatis into cases associated with chlamydial infection (C-AS, n = 68), cases associated with gonorrhoeal infection (G-AS, n = 19) and cases associated with neither of these sexually transmitted agents (NCNG-AS, n = 60).

No significant differences between these three groups were noted regarding reproductive history, previous salpingitis and lower genital tract infection (LGTI), as well as usage of contraceptive methods (IV). With respect to age, duration of pelvic pain before attending, ESR mm/h on admission, inflammatory reactions of the tubes as seen during laparoscopy and temperature $\geq 38^{\circ}\text{C}$ on admission, significant differences were noted between the three groups (IV). These differences are listed in Table 12.

There was no age difference between C-AS and G-AS patients (while NCNG-AS women were significantly older); C-AS women had significantly more often an afebrile illness than G-AS patients (which NCNG-AS women also had); the onset of pain was more evenly distributed throughout the menstrual cycle in the C-AS and NCNG-AS women than in the G-AS patients (unpublished results); complaints of increased vaginal discharge was as common among C-AS as among G-AS women (but the patients in these two groups complained of this significantly more often than the NCNG-AS women).

These three groups of salpingitis patients did not differ significantly from each other regarding symptoms of irregular bleeding or frequency and/or pain on voiding (IV).

The individual histories of 10 women, culture-positive for chlamydiae from the fallopian tubes, are reported in study V. The anamnesis, symptoms and signs and the laboratory findings varied considerably in these 10 women. Six of the patients were teenagers, while the oldest was 31 years old. The mean and the median duration of pelvic pain before attendance was 10.9 and 7 days, respectively, with a range of 3 to 27 days (V). The most consistent complaint, except pelvic pain, was increased vaginal discharge, which was reported by all but one of the women (V).

The ESR on admission ranged from 10 to 90 mm/h, only one patient had a "normal" ESR ≤ 15 mm/h. One of the patients had a febrile illness, and this woman did not harbour gonococci in the lower genital tract, which two of the other

Table 12. Significant differences between women with "chlamydial"-associated, "gonococcal"-associated and "nonchlamydial, nongonococcal". salpingitis

Parameter	C-AS=68		G r o u p		NCNG-AS=64		Groups compared	p
	n	%	n	%	n	%		
Age 20-24 years	28	41	8	42	15	23	C+G/NCNG	< 0.05
>30 "	7	10	3	16	18	28	C+G/NCNG	< 0.02
Pelvic pain 1-3 days	10	15	6	32	24	38	C/G+NCNG	< 0.01
ESR mm/h								
< 15	6	9	2	11	34	53		
16-30	18	26	11	58	18	28	$\chi^2 = 48.09, df 4$	< 0.001
> 30	44	65	6	32	12	19		
Inflammatory reactions of the tubes								
mild	15	22	5	26	34	53		
moderately severe	30	44	7	37	17	26	$\chi^2 = 14.94$	< 0.01
severe	23	34	7	37	13	20		
Increased vaginal discharge	51	75	14	74	37	58	C+G/NCNG	< 0.05
Temperature ≥ 38 °C	15	22	14	74	19	30	G/C	0.001
							G/NCNG	0.01

patients did (V). A tubal specimen for the culture of N gonorrhoeae was obtained from one of these two women, it yielded no growth (V).

The "clinical impression" of patients with C trachomatis was, that the course of their disease was comparatively "benign" notably towards the G-AS patients (IV,V). This "clinical impression" of C-AS patients has been reported by others (42).

In spite of the relatively benign clinical picture of the C-AS women in study IV, they had significantly more often raised ESR mm/h on admission, and moderately severe or severe inflammatory reactions of the tubes as seen during laparoscopy, compared with the G-AS and NCNG-AS patients (Table 12).

Apparently, some cases of C-AS run such a benign clinical course that they are not seen by doctors at all, or if they are, are misdiagnosed. In study V, one of the patients attended the gynecological out-patient clinic because of slight lower abdominal pain and irregular bleeding. The patient was discharged without treatment. A chlamydial culture obtained from the cervix at the time of this visit was positive. Due to a misunderstanding, the patient received no treatment. She returned three weeks later also complaining of increased vaginal discharge (V). On admission, the ESR was 90 mm/h, laparoscopy revealed a moderately severe inflammatory condition of the tubes (V).

In this context it is of interest that in a study of women infertile due to tubal pathology, C trachomatis was isolated from the fallopian tubes in patients with no history of salpingitis (60). Moreover, in serological studies of such infertile women, many of these patients had no history of PID (17,49, 77,92,131). Histological specimens obtained from the tubes in some of these women showed chronic inflammation (131). Several studies of women infertile because of tubal pathology have reported similar association between chlamydial antibodies and findings during laparoscopy and/or hysterosalpingography (5,17, 49,62,77,92,124).

SEQUELAE TO CHLAMYDIAL SALPINGITIS

General aspects

The most important sequela to AS is infertility. The only proof of a normally functioning fallopian tube is an intrauterine pregnancy, because post-infection could prevent the oocyte pick-up, even if the abdominal orificis are patent (127).

Fertility after "gonococcal" and "nongonococcal" AS, has been determined in three Swedish long-term studies (36,184,190). The overall infertility rates

after AS in these studies, after excluding voluntarily infertile women and couples with contributing infertility factors, varied between 19 and 25 % (36,184,190). In one study, a total of 159 women were evaluated after these exclusions (36). The patients were treated with penicillin combined with streptomycin, in addition half were given prednisolone while half received placebo (36). Fertility did not differ significantly between women with G-AS compared with NG-AS, nor did prednisolone therapy improve the fertility prognosis compared with placebo (36).

Significantly better fertility for women with G-AS compared with patients with NG-AS was, however, found in another long-term study (184). At the end of the follow-up period for these patients, 6 of 99 patients with G-AS had occluded tubes, while 26 of 150 patients with NG-AS had such occlusions ($p < 0.01$). The antibiotic therapy in this study consisted of either penicillin plus chloramphenicol, or penicillin plus streptomycin (184).

G-AS and NG-AS patients showed the same degree of fertility in a study comparing four different antibiotic regimens, i.e. penicillin combined with chloramphenicol, penicillin combined with streptomycin, ampicillin, or doxycycline (190).

However, women with NG-AS had a significantly higher risk of having an ectopic pregnancy, as the first pregnancy after the infection, compared with women with G-AS (190).

Mortality and ectopic pregnancies

In this material (studies I-VI), no deaths occurred related to the AS. From 1970 up to and including 1980, six cases of fatal salpingitis in women under the age of 40 years, have been reported in Sweden (112).

In study VI, no women with only one episode of AS, had an ectopic pregnancy with the first pregnancy after the infection. In women with two AS episodes during the observation period, two such pregnancies were reported (VI). Similar figures of ectopic pregnancies after AS have been reported previously (36). The observation time in study VI and in ref. 36, may have been too short to detect the incidence of ectopic pregnancies after one episode of AS. Other studies with longer observation times have found a higher frequency of ectopic pregnancies after AS (184,190).

Fertility/infertility after chlamydial salpingitis

As already mentioned (page 34) several studies have shown an association between infertility of tubal origin and antibodies to C trachomatis (5,17,49,62,

77,92,124,131). In one of the studies already mentioned, 9 of 23 women with sactosalpinx had no history of PID. Moreover the geometric mean titres of chlamydial antibodies in this sactosalpinx group was higher than in any other group studied (131). It has been suggested, that this correlation between tubal infertility and titres of antibodies to C trachomatis could be due to a subclinical chronic infection (61,62,77,92,131). If this is true remains, however, to be established. In this context, it is of interest, that C trachomatis has been isolated from 26 of 42 women with cornual tubal obstruction, while only two controls of 23 harboured the organism (5). According to Moore et al. (92), at least five of these isolations were made directly from the fallopian tubes.

In study VI, 299 women with laparoscopically verified AS and 49 controls were reviewed 2½ to 7½ years after the acute episode. In women exposing themselves to pregnancy, 50 (25.3 %) of 197 AS patients and 2 (6.7 %) of 30 control women were involuntarily infertile for at least one year ($p < 0.02$).

In women with the index infection as their first episode of AS, and excluding women who had had a double infection with both chlamydiae and gonococci isolated from the cervix, it was found that patients with G-AS and NCNG-AS had significantly milder inflammatory reactions of the tubes, than women with C-AS ($p < 0.05$) (IV). The fertility rate was, however, the same for women with only the index episode of AS, regardless of the findings with respect to different microbes. Thus 10 (22.7 %) of 44 women with C-AS, 4 (23.5 %) of 17 with G-AS, 2 (20.0 %) of 10 with CG-AS and 22 (22.0 %) of 100 with NCNG-AS were involuntarily infertile (VI).

Excluding couples with contributing infertility factors, such as oligomenorrhea or teratozoospermia, at most 19 % of the C-AS women were infertile due to tubal pathology. The corresponding percentages in the G-AS, CG-AS and NCNG-AS patients were 19 %, 11 % and 18 %, respectively (VI).

Thus, it does not seem that the fertility after C-AS differs from the fertility after other possible aetiological agents of AS.

Prognostic factors

Fertility was correlated to the number of AS episodes. Thus, at most 29 (17 %) of 162 women with one episode of AS, 9 (45 %) of 20 with two episodes and 3 of 6 (50 %) with three or more episodes of AS were involuntarily infertile due to tubal pathology ($p < 0.01$) (VI). Similar observations and figures have been reported previously (184).

In women with one episode of AS, fertility was correlated to the inflammatory reactions of the tubes (VI). Twelve (12.6 %) of 95 women with a mild in-

flammatory reaction of the tubes, 7 (17.9 %) of 39 with moderately severe and 10 (43.5 %) of 23 with severe inflammatory changes of the fallopian tubes were infertile, probably due to tubal pathology ($p < 0.02$) (VI). This correlation between the inflammatory changes of the tubes and the fertility prognosis has been reported previously (184,190).

In study VI, an ESR of ≤ 15 mm/h on admission, was found to be a favourable sign for the fertility prognosis, which is in agreement with other studies (36, 184).

Influence of contraceptive usage

Women who used OC's on admission and who had only had one episode of AS had no fertility problems. None of the 29 women, who were infertile probably due to tubal pathology, had used OC on admission, while 35 of the 133 fertile women had used this method of contraception ($p < 0.0004$) (VI). The use of OC during the salpingitis episode was also correlated with milder inflammatory changes of the tubes than in women using IUD's or other or no contraceptive methods ($p < 0.01$). In an extended series of 466 women with their first episode of AS, the correlation between the use of OC and the laparoscopically determined milder changes of the tubes was confirmed ($p < 0.01$) (unpublished data).

It has been shown, that the use of OC diminishes the risk of an ascending infection from the cervix compared with non-users of contraceptives, while the use of IUD increases this risk (27,153). That the use of OC also may diminish the inflammatory reactions of the tubes and is a favourable prognostic factor as to fertility after AS has not been reported previously.

Regarding the use of IUD's or other or no contraceptives on admission, no such correlations were found.

SUMMARY AND CONCLUSIONS

The annual number of acute salpingitis cases in Sweden has been estimated to be between 11,000 and 17,000. Directly and indirectly this costs Swedish society several hundred million crowns annually.

Isolation of C trachomatis from tubal specimens as the sole organism from the fallopian tubes in women with acute salpingitis (I,V) strongly indicates that chlamydiae are aetiological agents in this disease. Seroconversion and/or a significant change in titres of specific antibodies to C trachomatis during the acute episode of salpingitis is further evidence of the aetiological role of the organism in this disease (II,III,V).

The histopathological changes in the fallopian tubes in chlamydial salpingitis are characterized by a marked inflammatory infiltrate of lymphocytes and polymorphonuclear leukocytes through all layers of the tubal wall and a tubal exudate containing desquamated epithelial cells together with inflammatory cells (II).

Evidence of a current genital chlamydial infection was found in approximately 60 % of consecutively treated women with acute salpingitis (III). Thus in our hospital catchment region in southern Sweden, C trachomatis is currently the sexually transmitted aetiological agent most often linked to acute salpingitis (III).

Women with chlamydial salpingitis endure a longer duration of pelvic pain before seeking medical treatment and less often have a febrile illness on admission, than patients with other aetiologies of acute salpingitis (IV). On the other hand, in women with chlamydial salpingitis the ESR mm/h is usually higher and the laparoscopic condition of the tubes more often moderately severe or severe than in comparable women in whom C trachomatis cannot be diagnosed (IV). In the individual case, however, no conclusions can be drawn as to the aetiology on the basis of clinical signs and symptoms, or on laboratory data (V).

The proportion of women with unimpaired post-salpingitic tubal function seems to be the same, regardless of the aetiological agent of the disease (VI). The use of oral contraceptives at the time of the acute episode of salpingitis, appears to be a favourable prognostic factor, possibly because of significantly less severe inflammation of the tubes which was observed in patients using oral contraceptives as compared to those using other or no contraceptives (VI).

The present study has proved that in Southern Sweden, C trachomatis is currently the most important aetiological agent in a common disease, which often has serious implications for the future fecundity of the women afflicted.

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CHLAMYDIA TRACHOMATIS INFECTION IN PATIENTS WITH ACUTE SALPINGITIS

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Abstract We examined the prevalence of *Chlamydia trachomatis* in the cervix and the fallopian tubes of patients with acute salpingitis. Cycloheximide-treated McCoy cells were used as the growth medium. For purposes of comparison, women with infections confined to the lower genital tract and women without signs of genital infections were also studied. *C. trachomatis* was isolated from the cervix in 19 of 53 pa-

tients with acute salpingitis, in one of 18 lower-genital-tract infections and in none of 12 without signs of genital infection. *C. trachomatis* was recovered from six of the 20 valid specimens from the fallopian tubes of the patients with acute salpingitis. Our results indicate that chlamydia is a common etiologic agent in acute salpingitis. (N Engl J Med 296:1377-1379, 1977)

CHLAMYDIA trachomatis is a causative agent of sexually transmitted diseases, the frequency of which probably exceeds even that of *Neisseria gonorrhoeae*.^{1,2} Except for ocular infections, including neonatal conjunctivitis, little information has appeared on the role of *C. trachomatis* in complications of lower-genital-tract infections, including acute salpingitis.³⁻⁵

Acute salpingitis is a disease that is on the increase. Tubular occlusion, as a sequela, is the most common cause of involuntary childlessness in the female.⁶ In the majority of cases hitherto, the cause has not been established.

In our study, specimens for the culture of chlamydia were obtained from the cervix and the fallopian

tubes of patients admitted to the hospital with the presumed diagnosis of acute salpingitis and from clinically noninfected women. We found evidence that *C. trachomatis* is a common etiologic agent of acute salpingitis.

PATIENTS AND METHODS

Patients

Eighty-seven inpatients, 15 to 35 years of age, with the assumed diagnosis of acute salpingitis were studied at the Department of Obstetrics and Gynaecology, University Hospital, Lund. Forty of the 87 patients were using Cu-T 200 or Cu-7 200 intrauterine devices, and 21 were using oral contraceptives. Table 1 gives further gynecologic and obstetric anamnestic data. None of the patients studied had received antibiotic treatment before sampling.

Diagnostic laparoscopy was done within the day of admission. The laparoscopic technic and the criteria of a laparoscopic diagnosis of acute salpingitis have been described in detail elsewhere.^{7,8} The inflammatory changes of the fallopian tubes, as seen at laparoscopy, were graded as mild (reddened serosa on the tubes, edema of the tubal wall and purulent discharge from the abdominal orifice), moderate (more advanced inflammatory changes confined

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Table 1. Obstetric and Gynecologic Data and Laparoscopic Findings in 53 Patients with Acute Salpingitis, Related to Results of Cultures for *C. trachomatis* from the Cervix and from the Fallopian Tubes (20 Patients).*

GROUP	CERVIX		FALLOPIAN TUBES	
	POSITIVE CULTURES (19)†	NEGATIVE CULTURES (34)	POSITIVE CULTURES (6)	NEGATIVE CULTURES (14)
Never pregnant	8	11	3	4
Earlier episode:				
LGTI	6	10	4	3
Salpingitis	0	6	0	0
IUD user	9	15	3	5
OC user	6	5	2	3
Beginning of salpingitis after abortion or insertion of IUD	5	3	0	0
Laparoscopic grading:				
1	7	8	2	3
2	10	18	3	7
3	2	8	1	4

*LGTI represents lower-genital-tract infection, IUD intrauterine device, & OC oral contraceptives. 1 denotes mild, 2 moderate, and 3 severe (see text for definition of each grade)

†Figures in parentheses denote no. of patients.

to the tubes, such as tubal swelling, closure of the abdominal orifices and fibrin deposits on the serosal surfaces of the tubes) or severe (pelvic peritonitis or abscess formation). Of the 87 patients, 63 fulfilled the criteria of the laparoscopic diagnosis of acute salpingitis. Twenty-four patients had only signs of lower-genital-tract infection.

Controls

Twelve patients, matched for age with those with acute salpingitis, but without symptoms and signs of genital infection and subjected to laparoscopy for other reasons than assumed salpingitis, were studied as controls.

Sampling Technics and Transport Medium

We obtained specimens for *C. trachomatis* testing by rotating a sterile, cotton-tipped swab in the cervical canal and transported the specimens in sucrose-phosphate buffer,⁹ and specimens for *N. gonorrhoeae* from the cervical canal, the urethral orifice and the rectum with a cotton-tipped swab treated with charcoal and transported these specimens in modified Stuart's medium.¹⁰

We obtained specimens from the fallopian tubes and the pouch of Douglas under visual control during laparoscopy. For the chlamydial isolation studies, minute biopsy specimens were, in some cases, taken from the fimbriae of the abdominal orifice of the uterine tubes. In other cases, we took specimens for isolation of *C. trachomatis* and *N. gonorrhoeae* with the aid of a swab tipped with calcium alginate, which was fixed to a catheter and introduced through the abdominal orifice into the lumen of one of the tubes. We aspirated fluid from the pouch of Douglas through a baby-feeding catheter to which a syringe had been adapted.

Isolation Procedures

We isolated *C. trachomatis* on cycloheximide-treated McCoy cells.¹¹ The isolation and identification procedures used for *N. gonorrhoeae* were as reported elsewhere.¹²

RESULTS

Laparoscopically Verified Acute Salpingitis

Ten of the 63 cervical cultures from patients with acute salpingitis were spoiled by contaminating bacteria. *C. trachomatis* was isolated from the cervix in 19 of the remaining 53 patients (Table 2).

C. trachomatis was isolated from six of the 20 tubal specimens obtained, from biopsy specimens in three and from alginate swabs in three patients. *C. trachomatis* was not isolated from any of 29 cul-de-sac specimens.

Three of the patients from whom *C. trachomatis* was isolated from the uterine tubes were 15 to 19 years of age, and three were between 20 and 24.

Of the seven patients from whom *C. trachomatis* was isolated from the cervix and from whom tubal specimens were also obtained, the organism was recovered from the fallopian tubes in all but one.

Of the 19 patients with chlamydia-positive cervical cultures, five harbored *N. gonorrhoeae* in the cervix, as did six of the 34 chlamydia-negative cases. *N. gonorrhoeae* was isolated from the fallopian tubes of one of 14 patients. Two of these patients harbored gonococci in the cervix.

Signs of Lower-Genital-Tract Infection but No Laparoscopic Signs of Acute Salpingitis

Six of the 24 cervical cultures obtained were spoiled by contaminating bacteria. *C. trachomatis* was isolated from one of the remaining 18 specimens. *N. gonorrhoeae* was isolated from the cervix in two of the 24 patients. From these patients no tubal cultures were obtained.

No Genital-Tract Infection

None of the 12 specimens from the cervix yielded growth of chlamydia or gonococci. Five tubal specimens were obtained, but all were negative for *C. trachomatis*.

DISCUSSION

Since 1960 there has been a continuous increase in the annual number of cases treated because of acute salpingitis in our hospital catchment region. During the last few years the percentage of patients with acute salpingitis and cervical gonorrhea has decreased considerably. In 1968, the proportion of "gonorrheal" patients with salpingitis was approximately 50 per cent, whereas in 1974, it was less than 10 per cent.¹³

In a preliminary report of 12 patients with acute salpingitis (included in this series), *C. trachomatis* was isolated from the uterine tubes in two cases, and *N. gonorrhoeae* and *Mycoplasma hominis* were isolated in one case each.³ Acute-phase and convalescent-phase se-

Table 2. Isolation of *C. trachomatis* from Cervical Specimens of 53 Patients with Laparoscopically Verified Acute Salpingitis.

AGE GROUP	CHLAMYDIA ISOLATED	CHLAMYDIA NOT ISOLATED	TOTALS
yr	no. of patients		
15-19	6	11	17
20-24	9	8	17
25-29	1	8	9
30-34	2	5	7
35	1	2	3
Totals	19	34	53

rum specimens of these two patients were studied by a microimmunofluorescence technic for serotype-specific antibodies to *C. trachomatis*. In both patients there was a substantial increase in the antibody titer of both IgG and IgM antibodies to *C. trachomatis*, serotype ED, indicating an acute infection with this organism.

In previous series of patients with acute salpingitis we have studied tubal specimens for the presence of micro-organisms apart from chlamydia. Gonococci were isolated from the cervix in 134 out of 411 patients with acute salpingitis (32.6 per cent). We only isolated *N. gonorrhoeae* from the fallopian tubes of 17 of these 411 patients. In a previously reported series¹² of 50 patients with acute salpingitis *M. hominis* was isolated from the cervix in 31 (62 per cent), and from the fallopian tubes in four. For *Ureaplasma urealyticum* the corresponding figures were 28 and two. The isolation ratios for both *N. gonorrhoeae* and *M. hominis* from tubal versus cervical specimens of the same woman were approximately 1:10. This ratio could be compared with *C. trachomatis*, which we demonstrated in tubal material in six of seven cervical-culture-positive cases. As for gonococci and mycoplasmas, there was no relation between the isolation rate of *C. trachomatis* and the visual grading of the inflammatory changes of the fallopian tubes (Table 1).

In our study, the cultures taken from the tubal specimens were restricted to *N. gonorrhoeae* and *C. trachomatis* to obtain as much material as possible for the chlamydial culture study. Consequently, the possible coexistence of *C. trachomatis* and other organisms (except for gonococci) in the uterine tubes of patients with acute salpingitis could not be established. Not in a single case could both chlamydia and gonococci be isolated from the uterine tubes.

Material from the cul-de-sac, aspirated by culdocentesis, has been used for culture studies of acute salpingitis.⁵ However, our investigation indicates that material from the pouch of Douglas is not suitable for the isolation of *C. trachomatis*. Thus, this drawback provided still another reason for performing laparoscopy in patients with assumed acute salpingitis, for it simultaneously offers an opportunity for sampling from the uterine tubes.

Various antibiotic regimens have been recommended for the treatment of acute salpingitis, but since long-term follow-up studies are lacking, no particular treatment has been established as being of especial benefit. However, the findings of *C. trachomatis* in the fallopian tubes and the results of susceptibility tests of this organism¹⁴ suggest that antibiotics such as penicillins and aminoglycosides should be excluded in the treatment of acute salpingitis, especially when diagnostic facilities are lacking.

Tubal occlusion, as a sequela of acute salpingitis, is the most common cause of sterility in the female. In a long-term follow-up study (nine years or more) of women with acute salpingitis treated for approximately 14 days with penicillin G (benzylpenicillin) or chloramphenicol and streptomycin, the proportion of women with involuntary childlessness was 21.2 per

cent, whereas the corresponding figure in a series of age-matched women who had never suffered from salpingitis was 3.0 per cent.^{13,15} Because pregnancy is the only reliable means available for testing the function of the uterine tubes, evaluation of the fertility prognosis in patients with acute salpingitis treated with antibiotics, suitable for the treatment of *C. trachomatis* infection, will take eight to 10 years.

We have recently demonstrated an increased risk of acute salpingitis in women using intrauterine devices.¹⁶ We found no evidence that the use of such a device increases the risk of ascending gonococcal infections. We could not ascertain, from this study, whether the risk is increased in women infected with *C. trachomatis*. Nine of 19 patients who were culture positive for *C. trachomatis* in the cervix were using an intrauterine device, as were 15 of the 34 culture-negative patients.

The present study indicates the need for routine diagnostic cultures for *C. trachomatis* in women with signs of genital infection and suggests that prophylactic and epidemiologic measures, analogous to those used for *N. gonorrhoeae*, should be considered.

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Chlamydia trachomatis infection of the Fallopian tubes

Histological findings in two patients

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SUMMARY In two patients with acute salpingitis, *C. trachomatis* was isolated from the cervix. In one of the patients, the organism was also recovered from the Fallopian tubes, and in the other, chlamydial inclusions were found in Giemsa-stained tubal epithelial cells. A significant change in micro-immunofluorescence antibodies to *C. trachomatis* occurred in both patients during the course of the disease. The Fallopian tubes of both patients were removed and studied by conventional histological techniques and, in the case of one of them, by transmission electron microscopy.

Introduction

Chlamydia trachomatis is a common cause of sexually transmitted genital infections (Schachter, 1978). It can be recovered from the cervix of about one third of women with signs of cervicitis but from only a small percentage of healthy women (Hilton *et al.*, 1974; Oriel *et al.*, 1978; Ripa *et al.*, 1978).

In patients with laparoscopically verified acute salpingitis, *C. trachomatis* has been isolated from the cervix in 19 of 53 cases and from six of 20 tubal specimens from these patients (Mårdh *et al.*, 1977). Evidence of chlamydial infection in women with acute salpingitis has also been demonstrated by serological means (Treharne *et al.*, 1979).

In non-human primates experimental salpingitis with *C. trachomatis* has been reported (Ripa *et al.*, 1979).

In the present study two patients with acute salpingitis with evidence of tubal infection with *C. trachomatis* are reported. In both patients the tubes were removed and studied by histological techniques, and, in one of them, by transmission electron microscopy.

Patients and methods

SAMPLING TECHNIQUES

Specimens for the isolation of *C. trachomatis*, mycoplasmas, and ureaplasmas were obtained by rotating a sterile, cotton-tipped swab in the cervical canal and in the lumen of the uterine tubes. Specimens for isolation of gonococci were taken from the cervix, the urethra, and from the rectum with a cotton-tipped swab treated with charcoal.

TRANSPORT MEDIA

Specimens for the isolation of chlamydia were transported and stored at -20°C in a sucrose-phosphate buffer supplemented with $50\text{ }\mu\text{g}$ gentamicin and $2.5\text{ }\mu\text{g}$ amphotericin B per ml (Gordon *et al.*, 1969). The specimens for the isolation of mycoplasmas were transported in liquid B medium (Freundt *et al.*, 1979), and those for ureaplasmas in liquid S medium (Freundt *et al.*, 1979). The *Neisseria gonorrhoeae* specimens were delivered to the laboratory in a modified Stuart's medium (Gästrin *et al.*, 1968).

ISOLATION PROCEDURES

C. trachomatis was cultured using cycloheximide-treated McCoy cells (Ripa and Mårdh, 1977). The culture techniques for mycoplasmas, ureaplasmas (Freundt *et al.*, 1979) and *N. gonorrhoeae* (Mårdh

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and Weström, 1970) have been described in detail elsewhere.

SEROLOGICAL STUDIES

Serum antibodies to *C. trachomatis* were studied using a micro-immunofluorescence test (Treharne *et al.*, 1977). Fluorescein isothiocyanate conjugated rabbit anti-human anti-IgG and anti-IgM globulins (Dakopatt A/S, Copenhagen, Denmark) were used. The sera were titrated in two-fold dilutions; the lowest dilution used was 1/8.

Determination of antibodies to mycoplasmas and ureaplasmas was performed using an indirect haemagglutination test according to the method of Krogsgaard-Jensen (1971). Gonococcal anti-pilar antibodies were determined by an indirect haemagglutination test (Reiman and Lind, 1977).

HISTOLOGICAL TECHNIQUES

The tissue specimens were fixed in 10% formalin and processed by routine histological methods, including staining with haematoxylin and eosin, and with Giemsa.

TRANSMISSION ELECTRON MICROSCOPY

The specimens were fixed in 2.5% glutaraldehyde in

0.2 mmol/l cacodylate buffer, pH 7.4, for two hours. After repeated rinsing in the buffer, the specimens were refixed in 2% osmium tetroxide using the same buffer, dehydrated in ethanol and embedded in Vestopal W. Sections, 1 μ m thick, and were examined by phase contrast microscopy. Ultrathin sections were then cut on an LKA-Ultrathome, contrasted with lead citrate and uranyl acetate, and viewed in a Zeiss EM 10 electron microscope.

CASE REPORTS

Case 1

A 27-year-old white African woman (gravida 4, para 2, legal abortion 1) was referred for legal abortion in the tenth week of pregnancy. On bimanual palpation the uterus was enlarged, corresponding to the tenth week of pregnancy. No sign of genital infection was detected. Erythrocyte sedimentation rate (ESR) was 2 mm/h and white blood cell count (WBC) $11.0 \times 10^9/l$ with a normal differential count.

On 10 April 1978, legal abortion was performed by dilatation and suction curettage under general anaesthesia as an outpatient. No immediate complications were noted.

Two weeks later the patient was readmitted with low abdominal pain of three to four days' duration. General examination was normal. A moderate amount of brownish discharge was found in the vagina. The uterus was of



Fig. 1 Cross-section of Fallopian tube showing oedema and an intense infiltration of the epithelium, the subepithelial connective tissue, the muscular layer, and extending into the subserous layer (Case 1). Stained with haematoxylin and eosin, $\times 40$.

normal size and no adnexal masses were palpated. The pelvic examination caused moderate pain. Rectal temperature was 37.5°C, ESR 28 mm/h, and WBC $14.0 \times 10^9/l$. A clinical diagnosis of salpingitis was made, and the patient was treated in hospital with penicillin 10⁶ IU and streptomycin 500 mg twice daily for six days.

During the following three weeks the rectal temperature fluctuated between 37.5°C and 38.5°C. ESR increased to 64 mm/h. On repeated pelvic examinations increasing tenderness was noted and, therefore, 26 days after admission a laparotomy was performed. At operation both Fallopian tubes were swollen, about 5 cm in diameter. A purulent exudate poured from the mouth of the oviduct. On the right side there was a tubo-ovarian abscess while the left ovary was normal. Bilateral salpingectomy was performed. Ampicillin 2 g and metronidazole 1.2 g a day were given daily for six days postoperatively. The postoperative course was uneventful and the patient was discharged eight days after the operation.

Histological examination of the tubal specimens showed a marked inflammatory infiltrate through all the layers of the uterine tubes (Fig. 1). The lumina were filled with an exudate containing lymphocytes and desquamated epithelial cells. The epithelium, which was injured in some areas, was oedematous and infiltrated with inflammatory cells (Fig. 2). The muscle layers were infiltrated with

numerous lymphocytes and some polymorphonuclear leucocytes. The cellular infiltration extended into the subserous layer.

Giemsa-stained specimens examined by darkfield illumination showed intracytoplasmic inclusions in surface epithelial cells. The appearance of these inclusions was consistent with those found in *C. trachomatis* infected cells.

Cultures for gonococci and mycoplasmas from the tubes and cervix gave negative results. *U. urealyticum* was isolated from the cervix but not from the uterine tubes. *C. trachomatis* was recovered from the cervix the day before legal abortion was performed. At laparotomy five weeks later we failed to isolate *C. trachomatis* from the tubes. No chlamydial IgM or IgG antibodies were detected in serum samples taken the day before the therapeutic abortion. On day 41 after the abortion, the IgM micro-immunofluorescence antibody titre was 1/128 and that of IgG 1/32. Two months later the IgG titre was 1/128 while no IgM antibodies could be determined.

No IHA antibodies to mycoplasmas and ureaplasmas and no anti-pilar antibodies to gonococci were found before the abortion nor during the follow-up period.

Case 2

A 31-year-old Caucasian woman of Polish origin (gravida

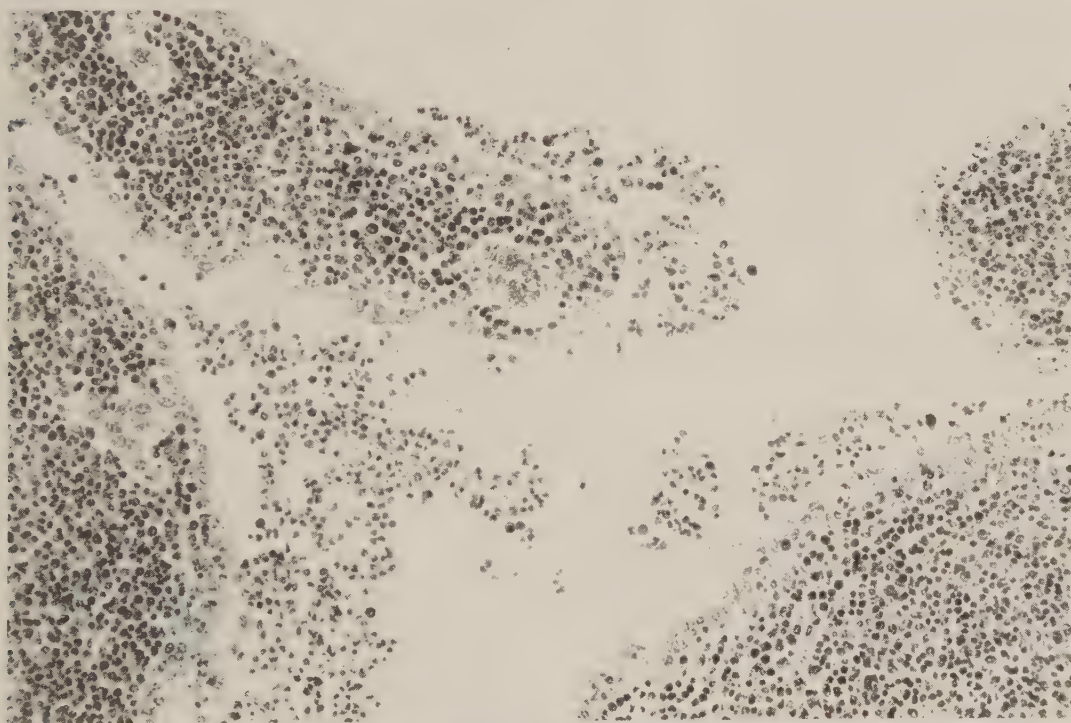


Fig. 2. Section of Fallopian tube showing the epithelium and the subepithelial tissue infiltrated with inflammatory cells. The epithelium is injured in some areas. The lumen contains inflammatory cells and desquamated epithelial cells (Case 1). Stained with hematoxylin and eosin, $\times 200$.

6, para 2, legal abortion 4) applied for legal sterilisation in January 1978 and was admitted on 20 April for the operation. After menstruating she had observed a yellowish discharge. Three days before admission she developed low abdominal pain. On admission her rectal temperature was 37.6°C, and ESR 40 mm/h. Vaginal examination showed a small amount of yellowish discharge with an equal number of vaginal epithelial cells and leucocytes in the wet smear. The uterus was of normal size but there were bilateral masses about 5 cm in diameter. The patient experienced moderate pain on pelvic examination.

A preoperative diagnosis of bilateral tubo-ovarian abscesses was made and, because the patient had applied for sterilisation, laparotomy was performed 26 hours after admission. No antibiotic treatment had been given before laparotomy.

At operation both Fallopian tubes were swollen, about 5 cm in diameter, with pus in the mouth of the oviduct. The tubal wall was about 5-10 mm thick. The ovaries were partly covered with a purulent discharge but no peritonitis was present. A bilateral salpingectomy was performed and doxycycline 200 mg was administered intravenously during the first 24 hours followed by 100 mg by mouth daily.

The postoperative course was uneventful and the patient left hospital on the sixth day after operation.

Histological examination of the tubes showed marked inflammatory changes through all layers of the tubes. The mucosal lining was very oedematous with inflammatory cells in the submucous and muscular layers to the serous lining. The lumen of the tube was full of leucocytes (Fig. 3).

By transmission electron microscopy a specimen from the epithelium of the Fallopian tubes was from one to several cells thick. There were ciliated and non-ciliated cells, both of which had surface microvilli and goblet cells at different stages of maturity lying interspersed among the ciliated cells and containing numerous vesicles filled with mucus. The cytoplasm of the goblet cells contained a granular endoplasmic reticulum. The nucleus of the ciliated and the non-ciliated cells varied in shape from round to oval or were greatly elongated. The nuclear membrane was often indented; the cytoplasm of these cells contained mainly agranular endoplasmic reticulum. The mitochondria, mainly oblong in form, were sometimes situated in the apical part of the cell. There were also lysosomes of different size both in the ciliated and non-ciliated cells. The apical part of these cells often protruded into the lumen (Fig. 4).

Cultures for gonococci gave a negative result. *C. trachomatis* was isolated from the cervix and the Fallopian tubes. Sera collected on day 21 postoperatively contained

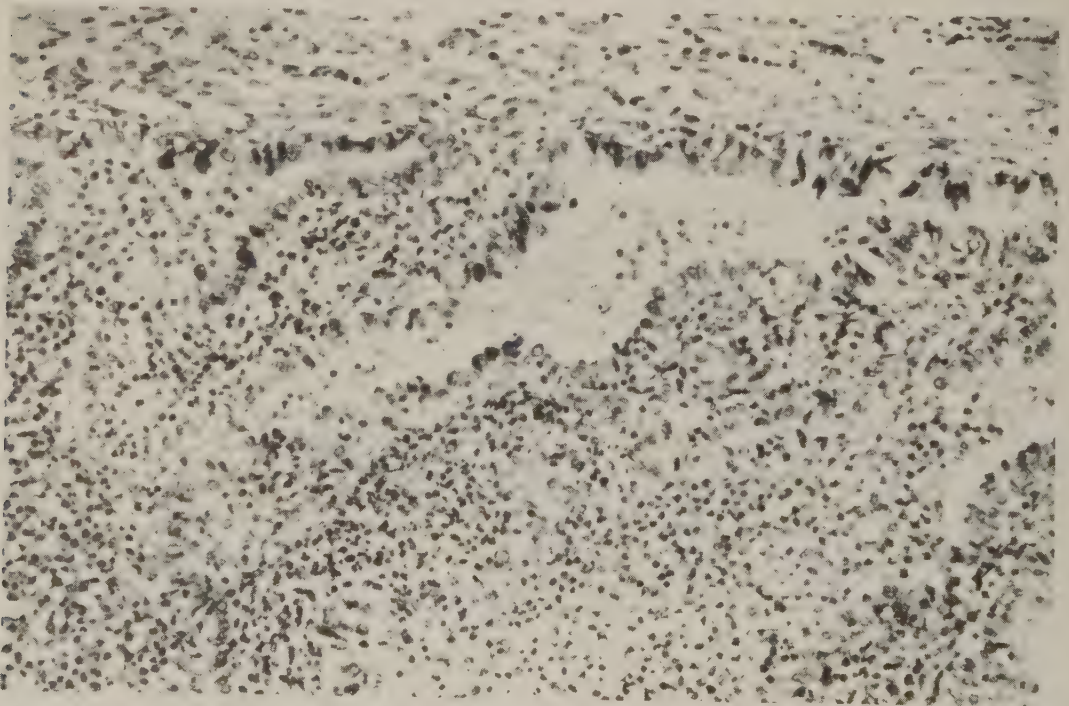


Fig. 3 Section of Fallopian tube. Marked inflammatory changes are evident throughout all the layers of the tube. The lumen contains leucocytes. The epithelium is oedematous and injured in some areas. Marked oedema with inflammatory cells in the submucous and muscular layers can be seen (Case 2). Stained with hematoxylin and eosin, $\times 200$.

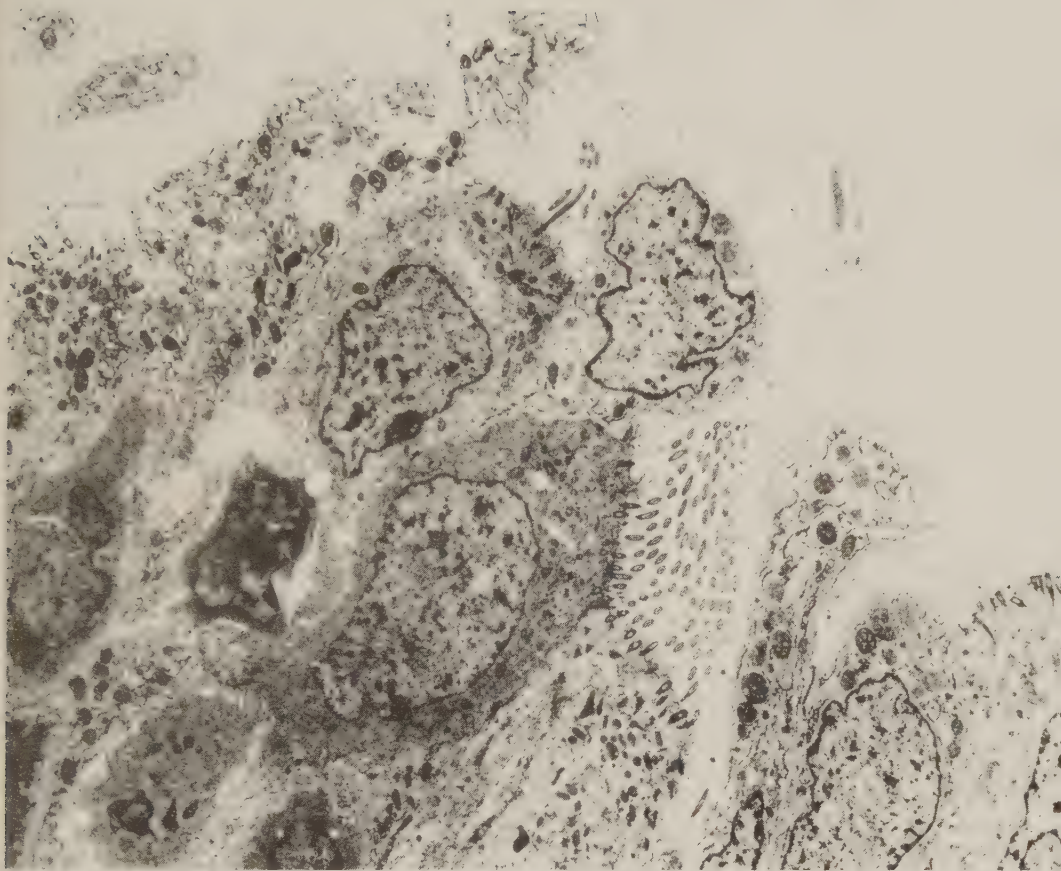


Fig. 4 Transmission electron micrograph of the surface epithelium of a Fallopian tube of a patient (Case 2) with acute salpingitis with evidence of *Chlamydia trachomatis* infection, $\times 4700$.

no IgM micro-immunofluorescence antibodies to *C. trachomatis* but IgG antibodies at a titre of 1/128. A second serum specimen was collected after a further month and contained IgG antibodies at a titre of 1/16 while no IgM antibodies were found. No anti-pilar antibodies to gonococci were found in the sera tested.

Discussion

Two main groups of non-tuberculous salpingitis have been recognised (Novak and Woodruff, 1974). In gonococcal salpingitis, the micro-organisms are spread from the lower genital tract through the lumen of cervical canal via the uterine mucosa to the tubes, which become red and swollen and contain a purulent exudate. Microscopically, the epithelium and the subepithelial tissue show an intense inflammation. In some areas the luminal surface epithelium is destroyed. Adhesion of the mucosal

folds are often seen. In nongonococcal salpingitis, the causative micro-organism is believed to gain entrance to the tissue through lesions in the cervical or endometrial epithelium and to spread to the parametria and tubes via blood vessels and lymphatics. The inflammatory swelling, which affects both the parametria and the tubes, is more pronounced than in gonococcal salpingitis. The swelling of the tubes is due to an oedematous thickening of the tubal wall. The tubal epithelium is usually intact and there is no exudate in the lumen.

Gonococci are seldom isolated from tubal specimens in women with acute salpingitis and cervical gonorrhoea (Mårdh *et al.*, 1978). The prognosis for fertility in antibiotic-treated cases of salpingitis with gonorrhoea is significantly better than in cases of salpingitis in which gonococci cannot be recovered (Weström and Mårdh, 1977). During the 1970s, the annual number of cases of

salpingitis with gonorrhoea in our hospital catchment region has been declining, even though the total number of such cases of acute salpingitis has increased during the same period (Weström and Mårdh, 1977). There is evidence that salpingitis caused by *C. trachomatis* is now more common in our area than that due to gonorrhoea (Mårdh *et al.*, 1977).

Experimental *C. trachomatis* salpingitis in grivet monkeys causes histological changes in the tubes which are similar to those of gonococcal salpingitis in man (Ripa *et al.*, 1979). *Mycoplasma hominis* produces changes which are similar to those in patients with nongonococcal salpingitis (Weström and Mårdh, 1977).

In Case 1, *C. trachomatis* was isolated from the cervix before abortion, at which time no antibodies to the micro-organism were detected. Two weeks after this operation, the patient was admitted to hospital with signs of acute salpingitis. She was treated with penicillin and streptomycin, which had no effect on the clinical course. Laparotomy was performed and both tubes were found to be swollen. Bilateral salpingectomy was performed. The histological changes in the tubes were the same as those described in textbooks for gonococcal salpingitis. Immunofluorescent studies showed intracytoplasmic chlamydial inclusions in some of the cells. IgM and IgG micro-immunofluorescence antibody titres to *C. trachomatis* were significantly raised.

Gonococci, mycoplasmas, and ureaplasmas could not be recovered from the tubes. The patient did not develop antibodies to any of these micro-organisms during the course of the disease. As in Case 1, therapeutic abortion may enable *C. trachomatis* to spread by way of the cervical canal to the tubes.

The other patient was admitted three days after the start of low abdominal pain. No diagnostic procedures had been performed before admission. When salpingitis was diagnosed, bilateral salpingectomy was performed as the patient had asked for legal sterilisation. Chlamydia were isolated from the Fallopian tubes and from the cervix whereas gonococci were not. During the postoperative period, a significant change in the titre of IgG antibodies to *C. trachomatis* but not to *N. gonorrhoeae*, *M. hominis*, or *U. urealyticum* was detected.

In those sections of the Fallopian tubes studied by transmission electron microscopy, no cellular inclusions consistent with those caused by chlamydia were noted. Ciliated cells could be seen but the cilia did not show any structural changes. The nucleus of the ciliated cells showed degenerative alterations with distorted morphology. The non-ciliated secretory

cells showed protrusions with plenty of microvilli indicating an increased luminal surface. In non-ciliated epithelial cells, an increased number of lysosomes suggesting an increased secretory activity was found.

The light microscopy findings of histological changes in the tubes from both patients were the same as those described in textbooks for gonococcal salpingitis (Novak and Woodruff, 1974). Furthermore, the inflammatory changes were similar to those in grivet monkeys experimentally infected in the tubes with *C. trachomatis* (Ripa *et al.*, 1979).

It seems that the old textbook differentiation of gonococcal and nongonococcal salpingitis ought to be re-evaluated.

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Antibodies to *Chlamydia trachomatis*, *Mycoplasma hominis*, and *Neisseria gonorrhoeae* in sera from patients with acute salpingitis

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SUMMARY Paired sera from 60 consecutive patients with acute salpingitis, confirmed by laparoscopy, were examined for serum antibodies to *Chlamydia trachomatis*, *Mycoplasma hominis*, and *Neisseria gonorrhoeae*. By a microimmunofluorescence (MIF) test IgM or IgG antibodies to *C. trachomatis* or both were present in sera from 80% of the patients; by indirect haemagglutination (IHA) tests antibodies to *M. hominis* and *N. gonorrhoeae* pillar antigens were present in 40% and 18% respectively. In a control group of 50 pregnant women antibodies to the same three organisms occurred in 8%, 8%, and 6%. Evidence of current chlamydial infection was found in 35 (58%) and of current gonococcal infection in five (8%) of the 60 patients by culture or serological tests or both. The results of chlamydial antibody tests correlated with the severity of the tubal inflammation (as shown by laparoscopy) and the duration of the lower abdominal pain before attendance. The predictive values of a positive and a negative MIF test result were 44% and 83% respectively and of the IHA gonococcal antibody test 36% and 100% respectively. Significant rises in titre of antibodies to *M. hominis* were found in 12% of patients. A four-fold or greater rise in titre indicated probable double infections with chlamydia and mycoplasmas in 7% of patients. Thus, at present gonococcal salpingitis appears to form only a small proportion of all cases of salpingitis in southern Sweden, and in patients with nongonococcal salpingitis infections with *C. trachomatis* and *M. hominis* commonly occur.

Introduction

Although there are diverse opinions¹⁻² on the proportion of acute salpingitis that is caused by *Neisseria gonorrhoeae*, most workers agree that other agents can also be responsible for this condition. Nongonococcal salpingitis may be caused by a variety of organisms, the relative importance of which, however, seems to vary in different regions.

Some workers^{1,3-5} have proposed that gonococcal infections account for the vast majority of tubal infections. Others have presented evidence that nongonococcal salpingitis is at least as common as

gonococcal salpingitis in their hospital catchment areas^{2,6-9} and that the proportion of nongonococcal cases has increased during the last decade.^{7,10}

We have shown that *Chlamydia trachomatis*^{7,11-13} and *Mycoplasma hominis*^{8,14,15} are agents that may cause nongonococcal pelvic inflammatory disease (PID). In the present study, we tested paired sera from consecutive patients with acute salpingitis for antibodies to *C. trachomatis* by an indirect immunofluorescence test and for antibodies to *M. hominis* and *N. gonorrhoeae* by indirect haemagglutination tests.

Patients and methods

STUDY POPULATION

The series of patients with salpingitis were 60 consecutive cases treated at the department of

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obstetrics and gynaecology at the University Hospital, Lund, Sweden, and were verified by laparoscopy¹⁶; 19 cases were classified as severe, 25 as moderately severe, and 16 as mild. Paired sera were obtained; the time elapsing between the collection of the two specimens varied from four to 60 days (mean 15.7 days). The ages of the patients varied from 14 to 48 years (mean 23.5 years). Ten of the patients had had one previous episode of salpingitis and one had had more than one.

CONTROLS

Sera from 50 pregnant women attending consecutively from the same hospital catchment area served as controls. Their ages ranged from 17 to 38 years (mean 23.8 years). Whether any of these women had previously had salpingitis was not known.

SEROLOGY

A modified microimmunofluorescence (MIF) test¹⁷ using three pools of antigen of *C trachomatis*—immunotypes A-C, D-K (except J), and LGV 1-3—was used to demonstrate both IgM and IgG antibodies.

Antibodies to *M hominis* were detected by an indirect haemagglutination test, which was in principle performed as described by Krogsgaard-Jensen.¹⁸ The antigen was prepared from strain D188 of *M hominis*, which had been isolated from the cervix of a patient with acute salpingitis. After centrifugation of sonically treated cells, the supernatant fluid was used to sensitise formalised sheep erythrocytes.

Gonococcal antibodies were determined by an indirect haemagglutination (IHA) test, using pillar antigens prepared from two strains of *N gonorrhoeae*.¹⁹

In the microimmunofluorescence tests, titres of ≥ 16 (IgM) and ≥ 32 (IgG) were considered to give positive results for antibodies to *C trachomatis*, while titres ≥ 40 were considered to give positive results in both indirect haemagglutination tests.

CULTURE

The techniques used for the isolation and identification of *C trachomatis* and *N gonorrhoeae* were those described.¹¹ Cervical specimens were cultured for *C trachomatis* and cervical, urethral, and rectal specimens for *N gonorrhoeae*. Cultures for *M hominis* were not regularly performed and no cultures were performed for the control group.

PREDICTIVE VALUES AND STATISTICAL CALCULATION

The predictive values (for the diagnosis of chlamydial and gonococcal infection) for positive and negative test results by the MIF test (IgG) and by the IHA test

for gonococcal pillar antibodies were calculated as described.²⁰ Kendall's Tau-C test* was used to determine the correlation between the severity of the tubal inflammatory changes, the duration of pelvic pain before attendance, and the MIF titre to *C trachomatis* on admission. In these determinations patients who seroconverted or had at least a four-fold rise in titre of IgM or IgG antibodies or both or had an IgM titre of ≥ 32 or IgG titre of ≥ 512 or both were considered to have a current chlamydial infection.

Results

ANTIBODY TITRES

The maximum titres for each patient of IgM and IgG antibodies to *C trachomatis* and of antibodies to *M hominis* and *N gonorrhoeae* are shown in tables I and II. Antibodies (IgG or IgM or both) to *C trachomatis* were detected in 80% of the patients and antibodies to *M hominis* and *N gonorrhoeae* in 40% and 18% respectively. IgM antibodies to *C trachomatis* occurred in 12 and IgG antibodies in all 48 seropositive patients. A significant change in antibody titre to *C trachomatis* occurred in 40% of the patients (21 with IgG, one with IgM, and two with both antibodies), to *M hominis* in 12%, and to *N gonorrhoeae* in 5% (table III).

The predictive values for a positive and a negative MIF test result were 44% and 83% respectively; those for the IHA gonococcal pillar antibody test were 36% and 100% respectively. These calculations were based on the assumption that the diagnosis of a current infection was equivalent to a positive culture result for the organism.

CULTURE AND SEROLOGY

The results of cultural and serological studies are compared in table IV. None of the 12 patients with IgM antibodies to *C trachomatis* was culture-positive for chlamydia. Evidence of a current infection (culture-positive or significant change of antibody titre or both) with *C trachomatis* and *N gonorrhoeae* occurred in 35 (58%) and five (8%) respectively. Of the 23 patients with positive cultures for chlamydia 17, five, and one had titres of < 64 , 128-256, and > 512 respectively. Ten had a significant rise in the titre of antichlamydial IgG antibodies.

The four patients who harboured gonococci were all in the group of 11 patients who had IHA pillar antibodies to *N gonorrhoeae*. Two of the three patients with a significant rise in titre harboured gonococci.

*In these calculations the diagnosis of chlamydial and gonococcal infection was based on the result of cultures from the lower genital tract.

Antibodies to *Chlamydia trachomatis*, *Mycoplasma hominis*, and *Neisseria gonorrhoeae*

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TABLE I Maximum MIF test titres of IgM and IgG antibodies to *Chlamydia trachomatis* in 60 patients with acute salpingitis

Ig class	No of patients with titre:									
	<8	16	32	64	128	256	512	1024	2048	4096 8192
M	48	2	2	1	5	1	1	0	0	0
G	11	1	5	14	7	4	8	2	4	3

TABLE II Maximum titres of antibodies to *Mycoplasma hominis* and *Neisseria gonorrhoeae* in 60 patients with acute salpingitis

Organism	No of patients with maximum antibody titre:				
	≤20	40	80	160	320
<i>M. hominis</i>	36	9	9	4	2
<i>N. gonorrhoeae</i>	49	3	4	2	2

TABLE III Number and percentage of 60 patients with salpingitis showing antibodies to *Chlamydia trachomatis*, **Mycoplasma hominis*, and *Neisseria gonorrhoeae*

Organism	No (%) of patients with antibody titre:		
	Static	Increased†	Total
<i>Chlamydia trachomatis</i>	26 (43)	22 (37)	48 (80)
<i>Mycoplasma hominis</i>	17 (28)	7 (12)	24 (40)
<i>Neisseria gonorrhoeae</i>	8 (13)	3 (5)	11 (18)

*IgM or IgG or both

†Significantly

TABLE IV Comparison of results of culture for *C. trachomatis* and *N. gonorrhoeae* and of serological tests on 60 patients with acute salpingitis

Culture result	No of patients‡	MIF* test result		No of patients§	IHA† test result	
		+	-		+	-
+	23	21	2	4	4	0
-	37	27	10	56	7	49
Total	60	48	12	60	11	49

*IgG antibodies

†Gonococcal pillar antibodies

‡Specimens (cervical) cultured for *C. trachomatis*§Specimens (urethral, cervical and rectal) cultured for *N. gonorrhoeae*

STATISTICAL ANALYSIS

There was a correlation between the severity of the tubal changes and the results of the MIF tests ($P < 0.005$) and between the duration of pelvic pain before attendance and the results of serological tests ($P < 0.009$).

Four (7%) of the 60 patients had a significant rise in antibody titres to both *C. trachomatis* and *M. hominis*. The three patients with a significant rise in the titre of IHA gonococcal pillar antibodies had no evidence of a current infection with chlamydia or mycoplasmas.

CONTROLS

Antibodies to *C. trachomatis*, *M. hominis* and *N. gonorrhoeae* were demonstrated in 8%, 8%, and 6% of the sera from the control group of pregnant women.

Discussion

The spectrum and relative importance of various aetiological agents of acute salpingitis are influenced by such factors as the relative proportion of cases of different severity and the proportion of venereal and iatrogenic cases (for example, tubal infections occurring after curettage, legal abortion, and insertion of an intrauterine contraceptive device). The proportions of patients with primary and repeated tubal infection may influence the outcome of microbiological studies, as may the mean time interval between the onset of symptoms and sampling.

In the present series there was about the same number of mild, moderately severe, and severe cases. Approximately 85% of the cases could be classified as venereal and 20% of the patients had had salpingitis before. As in all series of patients with acute salpingitis, the time between the onset of symptoms and attendance varied considerably (from one day to more than two weeks).

The aetiological pattern of acute salpingitis seems to show geographical differences. The percentage of cases associated with gonorrhoea in recent studies from the USA has been about twice that of similar studies from Europe.^{3 4 6 8 15 21} A change in the aetiological pattern with time has also been found in Lund, Sweden; in the middle 1960s about every second case of acute salpingitis had gonorrhoea whereas 12 years later less than 10% of all cases were infected with gonococci.¹⁰

The time interval between collection of the first and second serum specimen from some patients was comparatively short—less than one week. In others, the time between exposure to infection and sampling of sera was fairly long (more than several months in some cases). This, of course, limits the possibility of demonstrating evidence of a current infection by a significant change in titre. With regard to *C. trachomatis*, however, a correlation was found between a positive serological test result, as well as the antibody titre at initial examination, and the duration of the disease before attendance.

Recently developed tests^{19 20 22 23} for detecting gonococcal pillar antibodies have proved to be both sensitive and specific. Such antibodies may persist for years in patients treated for gonorrhoea.^{20 22} In the present series, gonorrhoea had been diagnosed earlier in only two patients, and gonococci could be recovered from only four of the 60 patients. Static,

low titres of IHA gonococcal pillar antibodies and negative culture results were found in six patients, suggesting an antibody response to a past rather than to a present gonococcal infection.

Recent experimental studies²⁴ in grivet monkeys indicate that chlamydia, like gonococci, can spread canalicularly to the Fallopian tubes and produce inflammatory changes that are similar to those found in women with gonococcal salpingitis.²⁵ In such experimental studies *M hominis* can spread to the parametria and produce parametritis and salpingitis.²⁶

In the present series of patients 40% had IHA antibodies to *M hominis*, while a significant rise in titre occurred in 12%. Cultures of *M hominis* were not regularly performed, but the serological test results suggest that current mycoplasmal infections had occurred in an appreciable number of the patients studied.

Of the three agents studied, antibodies occurred most often to *C trachomatis* (80%); 37% of patients had a significant rise in titre.

In experimental genital infection with *C trachomatis* in grivet monkeys, IgM antibodies to the organism were detectable between one and two weeks after the infection and had disappeared after a further five or six weeks.²⁷ Our experience so far suggests that even in deep-sited genital chlamydial infections in man, serum antibodies to the organism may persist for a limited period, provided adequate antibiotic therapy has been given. For example, patients with salpingitis with high titres of IgM and IgG antibodies to *C trachomatis*, and from whom the organism was recovered from the cervix and the Fallopian tubes (and who were treated with tetracycline), had no detectable antibodies two years later.²⁷ These findings might suggest that the vast majority of our patients in whom antibodies to *C trachomatis* were detected had a current or comparatively recent chlamydial infection.

Chlamydia were isolated more often in those patients with no detectable antibodies and in those with a low (32-64) rather than a high (>128) IgG antibody titre. No chlamydia were cultured from the patients with IgM antibodies to *C trachomatis*. These results indicate that an immune response to *C trachomatis* may result in negative culture results for the organism in cervical specimens. Antibodies in cervical secretions may also have a similar effect.

In the population studied, the serological "background noise" was low; antibodies to *C trachomatis*, *M hominis*, and *N gonorrhoeae* occurred in <8% of the 50 pregnant women tested as controls, who were all inhabitants of Lund. However, in an earlier study of sera from women living in Landskrona in the same county (Skåne),

chlamydial antibodies occurred in the cord blood of 25% of the infants of 139 puerperal women.²⁸ In a recent study of 300 consecutive gynaecological patients treated in the same department as the patients with salpingitis *C trachomatis*, *M hominis*, and *N gonorrhoeae* were isolated from the cervix of 6.1%, 23%, and 2.3% respectively (unpublished data).

In the present study serological tests suggested a concomitant current infection with both chlamydia and mycoplasmas in 7% of the 60 patients. None of the few patients with gonorrhoea had evidence of a current infection with either chlamydia or mycoplasmas. In the 300 outpatients referred to above, chlamydia occurred in 13% of the 23% who harboured mycoplasmas and in 29% of those with gonorrhoea. *M hominis* was recovered from 59% of those infected with *N gonorrhoeae*.

This study showed that acute salpingitis in Lund is associated with a current genital chlamydial infection in at least 40% of patients, whereas gonococcal infections at present occur in only a small percentage (8%) of all cases of salpingitis. Acute infections with *M hominis* could be detected in 10-15% of the patients with acute salpingitis in the area.

To obtain optimum conditions for establishing the diagnosis of chlamydial and gonococcal salpingitis, our study indicates that cultures as well as serological tests should be performed.

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Differences in some clinical and laboratory parameters in acute salpingitis related to culture and serologic findings

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Women with laparoscopically verified acute salpingitis (AS) were studied, and 151 were classified as having: chlamydia-associated AS (C-AS), gonococcal-associated AS (G-AS), and nonchlamydial, nongonococcal-associated AS (NCNG-AS). Patients with G-AS were more often febrile (rectal temperature $>38^{\circ}\text{C}$) and more often had a moderately elevated erythrocyte sedimentation rate (ESR) (16 to 30 mm/hr) compared to other patients. Women with NCNG-AS were more likely to have a normal ESR and a mild inflammatory reaction laparoscopically. C-AS women were more likely to have had pelvic pain for more than 3 days before seeking treatment and to have an ESR of >30 mm/hr on admission. Predisposing factors to AS, such as insertion of intrauterine device, hysterosalpingography, and curettage within 4 weeks of admission, were more common in the C-AS group. The tubal inflammatory changes in the C-AS group were generally more severe than expected from the relatively benign clinical course. (AM. J. OBSTET. GYNECOL. 138:1017, 1980.)

ACUTE PELVIC INFLAMMATORY disease traditionally has been divided into gonococcal and nongonococcal PID, based on the results of endocervical cultures for *Neisseria gonorrhoeae*.

Gonococcal-associated salpingitis differs from nongonococcal disease with respect to abnormal vaginal discharge, elevated temperature, and duration of pelvic pain on admission.^{1, 2} In several series of PID patients the isolation rates of gonococci from cervical specimens have varied considerably, viz., between 0% and 80%.^{3, 4}

In our hospital catchment region cervical gonorrhea in women with acute salpingitis (AS) has declined from 50% in 1965 to 16.7% in 1979. During recent years, cultural and serologic studies have indicated that *Chlamydia trachomatis* is a major etiologic agent in acute salpingitis.⁵⁻⁷ The aim of the present study was to evaluate whether the history, clinical pic-

ture, and/or laboratory tests such as the ESR in chlamydial salpingitis differed from those in gonococcal salpingitis and/or nongonococcal, nonchlamydial salpingitis.

Material and methods

Diagnostic procedures. Laparoscopy was performed within 24 hours of admission to the hospital. The criteria for selecting the patients for laparoscopy, the laparoscopic technique, and the criteria for the diagnosis of acute AS were those described earlier.¹ The inflammatory changes in the fallopian tubes were graded as mild, moderately severe, or severe, according to a description published elsewhere.⁸

The technique used for the diagnosis of *C. trachomatis* was that described by Mårdh and associates.⁵ *C. trachomatis* was cultured on cyclohexamide-treated McCoy cells.⁹ The method used for the isolation and identification of *N. gonorrhoeae* was that reported by Mårdh and colleagues.¹⁰ For the demonstration of serum IgM and IgG antibodies to *C. trachomatis*, a modified microimmunofluorescence technique was employed, using pools of chlamydial antigen.¹¹

Patient material. Two hundred and forty-five women with laparoscopically verified acute AS were examined; 94 of these patients were excluded from the

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Table I. Age distributions of women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

Age group (yr)	Salpingitis group						Groups compared	Difference (p)
	C (n = 68)		G (n = 19)		NCNG (n = 60)			
	No.	%	No.	%	No.	%		
≤19	23	33.8	6	31.6	19	29.7	All	NS*
20-24	28	41.2	8	42.1	15	23.4	C + G/NCNG	<0.05
25-29	10	14.7	2	10.5	12	18.8	All	NS
≥30	7	10.3	3	15.8	18	28.1	C + G/NCNG	<0.02
Median age	23		21		24			

*Not significant.

Table II. Duration of pelvic pain before admission in women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

Pelvic pain (days)	Salpingitis group						Groups compared	Difference (p)
	C (n = 68)		G (n = 19)		NCNG (n = 64)			
	No.	%	No.	%	No.	%		
1-3	10	14.7	6	31.6	24	37.5	C/G + NCNG	<0.01
4-6	19	27.9	5	26.3	10	15.6	All	NS*
7-9	11	16.2	4	21.1	13	20.3	All	NS
≥10	28	41.2	4	21.1	17	26.6	All	NS

*Not significant.

study. The 151 AS patients studied were divided into three groups: (1) patients with evidence of chlamydial (but not gonococcal) infection (C-AS), (2) patients with evidence of gonococcal (but not chlamydial) infection (G-AS), and (3) patients in whom neither chlamydial nor gonococcal infection was diagnosed (NCNG-AS).

C-AS. The chlamydial group comprised 68 patients. *N. gonorrhoeae* was not isolated from the cervix, the urethra, the rectum, or the fallopian tubes in these patients. Furthermore, they presented one or more of the following criteria: (1) a fourfold or greater change in titer of IgM and/or IgG serum antibodies to *C. trachomatis*, (2) a titer of IgM antibodies $\geq 1:32$ and/or IgG antibodies $\geq 1:512$ to *C. trachomatis*, and (3) *C. trachomatis* isolated from the fallopian tubes.

G-AS. This group comprised 19 women. These women fulfilled all three of the following criteria: (1) *N. gonorrhoeae* isolated from the cervix, the urethra, and/or the fallopian tubes, (2) *C. trachomatis* not isolated from the cervix, the urethra, and/or the fallopian tubes, and (3) titers of IgM and IgG antibodies to *C. trachomatis* of $<1:8$ and $<1:64$, respectively.

NCNG-AS. This group consisted of 64 patients. They fulfilled the following criteria: (1) *C. trachomatis* and *N. gonorrhoeae* not isolated from the cervix, the urethra, and/or the fallopian tubes and (2) titers of IgM and IgG antibodies to *C. trachomatis* of $<1:8$ and $<1:64$, respectively.

Patients excluded. The following patients were excluded:

1. From the C-AS group: patients from whom *N. gonorrhoeae* was isolated from the cervix, the urethra, the rectum, or the fallopian tubes (n = 8)
2. From the G-AS group: women from whom *C. trachomatis* was isolated from the cervix, the urethra, or the fallopian tubes (n = 7); patients with IgM antibodies to *C. trachomatis* of 1:8 to 1:16 and such IgG antibodies at a titer of 1:64 to 1:256, respectively (n = 12)
3. From the NCNG-AS group: patients with IgM and/or IgG antibodies to *C. trachomatis* at a titer of 1:8 to 1:16 and/or 1:64 to 1:256, respectively (n = 54)
4. Women from whom *C. trachomatis* was isolated from the cervix or the urethra, with IgM and/or IgG antibodies to *C. trachomatis* at a titer of $<1:8$ and $<1:64$, respectively (n = 13)

Anamnestic, laboratory and clinical data. The following data were recorded: age, gravidity, earlier episodes of AS (laparoscopically confirmed) and lower genital tract infection (LGTI), contraceptive method used, duration of pelvic pain before seeking treatment, complaints of increased vaginal discharge, irregular bleeding, urgency and/or pain at micturition, febrile illness (rectal temperature $>38^{\circ}\text{C}$) and erythrocyte sedimentation rate (ESR in millimeters per hour) on

Table III. ESR on admission in women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

ESR (mm/hr)	Salpingitis group						Groups compared	Difference (p)
	C (n = 68)		G (n = 19)		NCNG (n = 64)			
	No.	%	No.	%	No.	%		
<15	6	8.8	2	10.5	34	53.1	C/NCNG	<0.001
16-30	18	26.5	11	57.9	18	28.1	G/NCNG	<0.01
							C/G	<0.05
>30	44	64.7	6	31.6	12	18.8	NCNG/G	<0.05
							C/G	<0.05
							C/NCNG	<0.001

Table IV. Laparoscopic appearance of the tubes of women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

Inflammatory reactions of the tubes	Salpingitis group						Groups compared	Difference (p)
	C (n = 68)		G (n = 19)		NCNG (n = 64)			
	No.	%	No.	%	No.	%		
Mild	15	22.1	5	26.3	34	53.1	NCNG/C	<0.001
Moderately severe	30	44.1	7	36.8	17	26.6	NCNG/G	<0.05
Severe	23	33.8	7	36.8	13	20.3	All	NS
							All	NS

Table V. Distribution of some parameters in women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

	Salpingitis group						Groups compared	Difference (p)
	C (n = 68)		G (n = 19)		NCNG (n = 64)			
	No.	%	No.	%	No.	%		
Never pregnant	34	50.9	9	47.4	27	42.2	All	NS*
Previous LGTI	18	26.5	5	26.3	25	39.1	All	NS
Previous salpingitis	14	20.6	1	5.3	9	14.1	All	NS
Increased vaginal discharge	51	75.0	14	73.7	37	57.8	C + G/NCNG	<0.05
Irregular bleeding	27	39.7	5	26.3	19	29.7	All	NS
Urgency, frequency, and/ or pain at micturition	11	16.2	3	15.8	12	18.6	All	NS
Contraceptive method used								
IUD	28	41.2	11	57.9	22	34.4	All	NS
OC†	16	23.5	3	15.8	11	17.2	All	NS

*Not significant.

†Oral contraceptive.

admission, and the degree of inflammatory changes in the fallopian tubes.

Predisposing factors for AS, such as insertion of an intrauterine device (IUD), curettage, or hysterosalpingography (HSG) within 4 weeks of admission, were recorded also.

Statistical methods. The Yates correction of the chi-square test and Fischer's exact test were used.

Results

Teenagers made up approximately one third of the patients in each of the three AS groups (Table I). In the

group aged 20 to 24 years, significantly more women belonged to the C-AS and G-AS groups than to the NCNG-AS group. More women were >30 years of age in this latter group than in the two other groups.

The patients in the G-AS and NCNG-AS groups more often had a short (<3 days) period of pelvic pain before seeking treatment compared to those in the chlamydia group (Table II). A high ESR (>30 mm/hr) was noted significantly more often in the C-AS group than in the other two groups (Table III). A normal ESR (<15 mm/hr) was observed significantly more often in the women in the NCNG-AS than in the C-AS and

Table VI. Relation between the titer of serum IgG antibodies to *C. trachomatis* and isolation of the organism from the cervix in women with chlamydial associated salpingitis

Titer	<i>C. trachomatis</i>		
	Isolated (n = 23)	Not isolated (n = 26)	Difference (p)
≤64	12	5	<0.05
128-256	6	4	NS*
≥512	5	17	<0.01

*Not significant.

G-AS groups. Mild inflammatory changes in the fallopian tubes were significantly more common in the NCNG-AS group than in the C-AS and G-AS groups (Table IV). In the G-AS group, 14 of 19 patients (73.7%) had febrile illness (rectal temperature $>38^{\circ}\text{C}$) on admission. The corresponding figures in the C-AS group were 15 of 68 (22.1%) and in the NCNG-AS group, 19 of 64 (29.7%). These differences were significant ($p < 0.001$ and $p < 0.01$, respectively).

No significant differences between the three groups studied were observed regarding previous AS and LGTI or the type of contraceptive used at the time of infection (Table V). An increased vaginal discharge was reported by significantly more patients in the C-AS and G-AS groups than in the NCNG-AS group.

Insertion of an IUD, curettage, or HSG within 4 weeks of admission was significantly more common in the C-AS group than in the other two groups ($p < 0.05$). Such a measure had been undertaken in 17.3% of the C-AS patients and in 6.3% of the NCNG-AS patients, whereas none of the G-AS women had undergone such measures.

In the C-AS group there was an inverse relationship between the serum IgG antibody titer to *C. trachomatis* on admission and the isolation of this organism from the cervix (Table VI). *C. trachomatis* was isolated significantly more often from those patients who, on admission, had an IgG antibody titer of $\leq 1:64$ than in those with a titer of $\geq 1:512$.

Comment

To establish the microbiologic etiology of acute salpingitis by using culture and/or serologic techniques is time-consuming and cannot be performed in all instances. Therefore the clinician's initial therapeutic decisions often must be based on "bedside" clinical findings. Because chlamydia salpingitis is so common^{5, 7} it seemed worthwhile to evaluate whether the clinical picture and/or laboratory results presented by women with C-AS differed from those of G-AS and NCNG-AS

patients. In order to present, insofar as possible, patients with evidence of purely chlamydial, purely gonococcal, and neither chlamydial nor gonococcal etiology for their infections, 94 (38.4%) of the cases were excluded. In these 94 patients the etiologic findings were equivocal.

The 151 women studied were divided into 68 cases with evidence of chlamydial etiology, 19 with gonococcal etiology, and 64 with neither chlamydial nor gonococcal etiology. The women with G-AS had febrile illness more often than the patients in the other groups and the duration of pelvic pain before seeking treatment was shorter than that for the C-AS group. These findings accord with earlier findings on gonococcal vis-à-vis nongonococcal salpingitis.^{1, 2}

The patients in the NCNG-AS group differed in several respects from the women in the other two groups. Their median age was greater, one out of four was 30 years of age or older. They did not report increased vaginal discharge as often as the patients in the C-AS and G-AS groups. Half of these women had a mild inflammatory reaction of the tubes and half had a normal ESR on examination.

The women who belonged to the C-AS group were younger than the NCNG-AS patients, but were older than the G-AS women. C-AS patients generally had experienced a more benign clinical course compared to G-AS and NCNG-AS patients. Women with chlamydial AS had a febrile illness less often and generally sought treatment after a longer period of pelvic pain than the women in the other two groups. On the other hand, the ESR on admission was highest in the C-AS group and the inflammatory changes in the fallopian tubes, as seen at laparoscopy, generally were more severe than expected from the relatively benign clinical picture. In this context, the findings of Henry-Suchet and Lofredo¹² are of interest. They isolated *C. trachomatis* from the fallopian tubes of women who had no history of salpingitis, but presented with tubal occlusion and signs of a "chronic active" tubal infection. Apparently, some cases of C-AS follow such a benign clinical course that they are not seen by doctors at all or, if so, only exceptionally at the hospital emergency room. This is of importance because of the destructive late effects on tubal function exerted by the chlamydial infections.

The clinical and laboratory appearance of the 94 women excluded from the study varied. The eight patients who fulfilled the criteria laid down for the C-AS group, but in whom *N. gonorrhoeae* was isolated from the cervix, appeared to be an intermediate group between the C-AS and the G-AS groups. These eight women had a short duration of pelvic pain and high ESRs. The 54 women with IgM and/or IgG antibody

titers to *C. trachomatis* between 1:8 and 1:16 and/or 1:64 and 1:256, respectively, appeared as a mixture of the C-AS and the NCNG-AS patients with regard to age, duration of pelvic pain, ESR on admission, and degree of inflammatory changes in the fallopian tubes as seen at laparoscopy.

Of the total 245 women, *N. gonorrhoeae* was isolated from the cervix in 46 (18.7%). *C. trachomatis* was isolated from the cervix in 67 (33.8%) of 198 representative specimens. Both *C. trachomatis* and *N. gonorrhoeae* were isolated from the cervix in 10 patients.

Predisposing factors such as IUD insertions and curettage were more common in patients with C-AS than in the other two groups. This indicates that an uncomplicated cervical chlamydial infection might be overlooked more easily than, for instance, gonococcal cervicitis. Because chlamydial cervicitis is generally more prevalent than gonococcal cervicitis¹³ and also

presents more uncharacteristic signs of infection,¹⁴ great care in excluding a chlamydial infection must be taken by all doctors performing procedures that could cause ascending spread of an infection of the lower genital tract.

On the basis of the clinical findings in cases of suspected salpingitis, one cannot with any confidence predict the etiologic agent in the individual case. The clinical and laboratory findings could, however, to a certain extent, guide the clinician in his choice of the initial diagnostic and therapeutic considerations. In this study, it was found that acute salpingitis caused by *C. trachomatis* often runs a comparatively benign clinical course. It is, therefore, of importance for all doctors who encounter such patients to be aware of the possibility of tubal chlamydial infection, to institute adequate antibiotic treatment, and to perform an epidemiologic follow-up of the patient's sexual partner(s).

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Acute Salpingitis with *Chlamydia trachomatis* Isolated from the Fallopian Tubes: Clinical, Cultural, and Serologic Findings

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Chlamydia trachomatis was recovered from the fallopian tubes of ten women with acute salpingitis. The median age of the patients was 19 years. The duration of pelvic pain before consulting a physician ranged from three to 27 days (median, seven days). Half of the patients complained of irregular bleeding, and nine reported increased vaginal discharge. One patient had a rectal temperature of $>38^{\circ}\text{C}$, and one had an erythrocyte sedimentation rate of <15 mm/hr. At laparoscopy, mild inflammatory changes were seen in the tubes of three patients, five had moderately severe inflammation, and two had pelvic peritonitis. *C. trachomatis* could not be isolated from the cervix of two patients. Paired sera were available from eight patients, six of whom had a significant rise in titer of IgG antibodies to *C. trachomatis*. Two women had IgM antibodies. Two other women, who harbored *Neisseria gonorrhoeae* in the cervix, had antibodies to gonococcal pili; one had a significant decrease in titer. This latter patient was one of the patients with a stationary titer of antibodies to *C. trachomatis*. One patient had a stationary titer of antibodies to *Mycoplasma hominis*. In general, chlamydial salpingitis seems to have relatively benign symptoms. Neither the failure to isolate *C. trachomatis* from the cervix nor a stationary titer of antibodies to the organism precludes a chlamydial etiology of acute salpingitis.

RECENT STUDIES, particularly those from the Scandinavian countries and the United Kingdom, have shown that *Chlamydia trachomatis* is an important etiologic agent of acute salpingitis.¹⁻¹¹ Patients with *Chlamydia*-associated salpingitis differ clinically with respect to duration of pelvic pain, erythrocyte sedimentation rate on admission, and frequency of febrile illness, from patients who have salpingitis associated with gonorrhea.¹⁰ However, the histopathological changes in the fallopian tubes are similar in chlamydial and gonococcal salpingitis.⁴

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This study presents individual findings for ten women with acute salpingitis. All had *C. trachomatis* isolated from the fallopian tubes.

Material and Methods

Patients

Ten women with acute salpingitis who had *C. trachomatis* isolated from the fallopian tubes were studied. Laparoscopy was used for diagnosis and procurement of specimens. Laparoscopy was performed within 24 hr of admission and before any treatment had been instituted. The laparoscopic technique and the criteria used for the classification of the tubal inflammatory changes as mild, moderately severe, and severe have been published¹² and can be described briefly as follows. (1) Mild inflammation indicates that tubes are reddened, swollen, and covered by purulent discharge. (2) "Violin-string" adhesions indicates that fibrin deposits are present, but tubes are freely movable and the abdominal ostia are open. (3) Moderate inflammatory changes are similar to those described as mild, but the changes are more marked; the tubes are not freely movable, and patency of the abdominal ostia is uncertain. (4) Severe inflammation indicates pelvic peritonitis and/or abscess formation with closed ostia.

Clinical Data

The following data were recorded: age; history of previous pregnancies (if any) and of previous infections of the lower genital tract or salpingitis; contraceptive method used; duration of pelvic pain before consulting a physician; and complaints of increased

TABLE 1. Clinical Data for Ten Women Who Had *Chlamydia trachomatis* Isolated from the Fallopian Tubes

Patient No.	Age (years)	Previously Pregnant	Previously Treated for LGTI*	Use of		No. of Days of Abdominal Pain before Admission
				Intrauterine Device	Oral Contraceptive	
1	19	+	+	+	—	4
2	18	—	+	—	—	7
3	20	+	—	—	+	27
4	24	—	—	+	—	14
5	19	—	—	—	+	26
6	31	+	+	—	—	5
7	17	—	+	—	+	3
8	17	—	—	—	—	3
9	22	+	+	+	—	13
10	18	+	+	+	—	7

* LGTI = Lower genital tract infection.

vaginal discharge, irregular bleeding, urgency, and/or pain on voiding.

In addition, the erythrocyte sedimentation rate (mm/hr) and the rectal temperature on admission were recorded.

Sampling and Culture Techniques

Both the techniques for sampling and the sites sampled have been described,^{2,14} (except for the attempts to isolate *C. trachomatis* from the urethra, which was done by rotation of a sterile cotton-tipped swab in the urethral orifice), as have the methods for the isolation and identification of *C. trachomatis*,¹⁴ *Neisseria gonorrhoeae*,¹⁵ and *Mycoplasma hominis*.¹³ The method of Holst et al. were used for the study of bacteria (apart from *N. gonorrhoeae*).¹⁶

Serologic Methods

For the demonstration of IgM and IgG antibodies to *C. trachomatis* in serum, a modified microimmuno-

fluorescence technique was used.¹⁷ An indirect hemagglutination (IHA) test was used for the detection of antibodies to *N. gonorrhoeae*¹⁸ and *M. hominis*.¹⁹

Results

The median age of the patients was 19 years (range, 17–31 years). Half of the women were nulligravidae. Four had an intrauterine device, and three used oral contraceptives (combination tablets) (table 1).

One woman (patient no. 1) had been treated for uncomplicated gonorrhoea two years previously. Earlier episodes of salpingitis were reported by one woman (patient no. 6), and six patients had been treated earlier for infection of the lower genital tract (table 1).

The mean and the median durations of pelvic pain before admission were 10.9 and 7.0 days, respectively (table 1). All but one patient reported increased vaginal discharge. Five women complained of irregular bleeding and two of frequency and/or pain on voiding. At admission one patient had an erythrocyte

TABLE 2. Some Symptoms and Signs in Ten Women Who Had *Chlamydia trachomatis* Isolated from the Fallopian Tubes

Patient No.	Increased Vaginal Discharge	Irregular Bleeding	Frequency and/or Pain on Voiding	Febrile on Admission*	ESR (mm/hr)†	Tubal Inflammatory Changes		
						Mild	Moderately Severe	Severe
1	+	+	+	—	45	...	...	+
2	+	—	—	+	45	+	...	...
3	+	+	—	—	10	...	+	...
4	+	—	—	—	35	...	+	...
5	+	+	—	—	90	...	+	...
6	+	—	—	—	40	...	...	+
7	+	+	—	—	27	...	+	...
8	+	+	—	—	16	+	...	...
9	+	—	—	—	29	+	...	...
10	—	—	+	—	45	...	+	...

Febrile = rectal temperature of > 38°C.

† ESR = erythrocyte sedimentation rate.

sedimentation rate of <15 mm/hr (table 2). Half of the patients had moderately severe inflammatory changes of the tubes, three had mild changes, and two had pelvic peritonitis (table 2).

Seven of the patients had *C. trachomatis* isolated from the urethra and/or the cervix (table 3). *C. trachomatis* had been isolated from the cervix of another patient (no. 5) three weeks before admission. At that time she was treated for acute otitis media with phenoxymethylpenicillin (800 mg four times daily). Two cervical specimens taken at admission from this patient proved negative for *C. trachomatis*, but chlamydiae were isolated from the fallopian tubes.

Two patients had *N. gonorrhoeae* isolated from the cervix, but this organism could not be recovered from any of seven tubal specimens studied (table 3). Cultures for aerobic, facultative anaerobic, and strictly anaerobic bacteria were inoculated with specimens from the tubes and/or exudate from the cul-de-sac of five of the patients; all were negative. Specimens from the fallopian tubes of four patients were cultured for *M. hominis*; all were negative. *M. hominis* was recovered from the cervix of one of these patients (no. 1).

A fourfold or greater rise in the titers of IgG antibodies to *C. trachomatis* was demonstrated in six of the eight paired sera available (table 4). The two remaining patients (no. 7 and 8), in whom a significant change in titer of antibody to *C. trachomatis* could not be demonstrated, both had a concomitant infection with *N. gonorrhoeae*. These two patients also had antibodies to *N. gonorrhoeae*, as shown by the IHA test; one had a stationary titer, while the other had a titer that decreased significantly. Antibodies to

TABLE 4. Results of Tests for Antibodies to *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma hominis* in Sera from Women Who Had *C. trachomatis* Isolated from the Fallopian Tubes

Patient No.	Interval between serum samples*	Titer of Antibodies to			
		<i>C. trachomatis</i>		<i>N. gonorrhoeae</i>	<i>M. hominis</i>
		IgM	IgG		
1	0	16	512	40	—
	8 days	128	2,048	ND†	ND
2	0	—	16	—	80
	6 days	—	128	—	80
3	0	—	256	—	—
	6 days	—	1,024	—	—
4	0	—	16	—	—
	12 days	—	512	—	—
	2.5 months	—	256	—	—
5	0	—	512	—	—
	11 days	—	4,096	—	—
	1 month	—	2,048	—	—
6	0	—	128	—	—
	1 month	—	16	—	—
	14 months	—	—	ND	ND
7	0	—	32	160	—
	5 days	—	64	160	—
8	0	—	32	320	—
	11 days	—	32	80	—
9	0	—	64	—	—
10	0	32	128	—	—

Note. The presence of antibodies to *C. trachomatis* was tested for by a microimmunofluorescence test; antibodies to the other two agents were tested for by indirect hemagglutination tests. A minus sign indicates a titer of <16 for IgM and <32 for IgG antibodies to *C. trachomatis*, and of <40 for antibodies to *N. gonorrhoeae* and *M. hominis*.

* Zero indicates sample obtained on admission to hospital.

† ND = not determined.

TABLE 3. Results of Cultures of Cervical, Urethral, and Fallopian Tube Specimens from Ten Women with Acute Salpingitis for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Patient No.	<i>C. trachomatis</i>			<i>N. gonorrhoeae</i>		
	Cervix	Urethra	Fallopian Tubes	Cervix	Urethra	Fallopian Tubes
1	S*	ND†	+	—	—	—
2	+	—	+	—	—	ND
3	+	—	+	—	—	—
4	+	+	+	—	—	—
5	—	—	+	—	—	—
6	+	ND	+	—	—	—
7	+	—	+	+	+	ND
8	+	+	+	+	—	—
9	+	—	+	—	—	ND
10	—	ND	+	—	—	—

* S = cell culture spoiled.

† ND = culture not done.

gonococcal pili were also found in the only patient (no. 1) with a history of uncomplicated gonorrhea. One woman (no. 2) had a stationary titer of IHA antibodies to *M. hominis*. No attempt to isolate *M. hominis* from this patient had been made.

Discussion

A chlamydial etiology in a case of acute salpingitis is suggested if *C. trachomatis* is isolated in pure culture from the fallopian tubes. We have never recovered this organism from normal tubes.² Recently, it was reported that *C. trachomatis* might also be recovered from the tubes of women with chronic pelvic inflammatory disease.²⁰

Chlamydiae were recovered from the tubes, but not

from the cervix, of two of our patients. This pattern is in contrast to that with *N. gonorrhoeae*, which generally can be recovered from the cervix if it is present in the fallopian tubes.²¹ This discrepancy may be due to differences in local immunologic factor(s) that would render it more difficult to recover chlamydiae from cervical than from tubal specimens.

In the present study, all women had serum IgG antibodies to *C. trachomatis*. In six of the eight patients from whom more than one serum specimen was obtained, a significant change in the titer was demonstrated. In one of the remaining two women, in whom the titers recorded differed by only one dilution, the interval between the drawing of the serum specimens was only five days.

Six of the patients had been treated for lower genital tract infection before being taken ill with salpingitis. These infections might have been associated with *C. trachomatis*, and this association could explain why only two women had IgM antibodies to *C. trachomatis*.

The two women who had *N. gonorrhoeae* isolated from the cervix had the shortest duration of pelvic pain on admission, a fact which is in accord with findings of other investigators.²² Of the three patients with antibodies to *N. gonorrhoeae*, only patient no. 1 had evidence of previous gonorrhea, which on that occasion was not associated with salpingitis. The other two patients with antibodies to gonococcal pili both harbored *N. gonorrhoeae*; one had a significant decrease in the titer.

Only one patient had antibodies to *M. hominis*, and the titer did not change. No attempt was made to isolate *M. hominis* from this patient. In contrast to *C. trachomatis* and *N. gonorrhoeae*, *M. hominis* seems mainly to cause parametritis among patients with signs of pelvic inflammatory disease (authors' unpublished data).

The history as well as the clinical and laboratory findings for the patients in the present study showed great diversity. The two women with pelvic peritonitis were among those with only brief pelvic pain. The patients with the highest and lowest erythrocyte sedimentation rates on admission both had moderately severe inflammation and both had suffered pelvic pain for more than three weeks.

On admission, women with *Chlamydia*-associated salpingitis have more often suffered pelvic pain for more than three days than women with gonococcal or nongonococcal, nonchlamydial salpingitis.¹⁰ Fever (rectal temperature >38°C) is more frequent in gonococcal than in nongonococcal salpingitis.^{22,23} In this study, only one patient had a febrile illness. An erythrocyte sedimentation rate of >30 mm/hr on admission is

more common in chlamydial than in nonchlamydial salpingitis.¹⁰ This difference might be explained by the more insidious onset of symptoms in chlamydial salpingitis. It also causes the patient to wait for a longer time after the onset of pelvic pain before consulting a physician. Six of the women in the present study had an erythrocyte sedimentation rate of >30 mm/hr. The two women with a concomitant gonococcal infection had shortlived pelvic pain and an erythrocyte sedimentation rate of <30 mm/hr on admission. In general, the relatively benign clinical course reported for *Chlamydia*-associated salpingitis¹⁰ was verified in this series of patients from whose fallopian tubes *C. trachomatis* was isolated.

Recent studies indicate that infections with *C. trachomatis* are common in women with signs of salpingitis.^{2,3,5,6,10,11} Thus the organism was isolated from the cervix of 28 (26%) of 106 consecutive patients with acute salpingitis.⁵ Serologic tests suggested a current chlamydial infection in 66% of 143 women with laparoscopically verified salpingitis in one study,⁶ and 57% of 206 women in another study.¹¹

This study shows that neither a cervical culture negative for *C. trachomatis* nor stationary titers of antibodies to *C. trachomatis* are inconsistent with the presence of *C. trachomatis* in the fallopian tubes. The physician must be aware of these facts when choosing antibiotic therapy for patients with acute salpingitis. Since 1978 we have treated women with acute salpingitis with doxycycline (200 mg daily for ten to 14 days). All male consorts of *Chlamydia*-positive patients are requested to contact the clinic for venereal diseases.

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Infertility after acute salpingitis - with special reference to Chlamydia trachomatis

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Of 552 women with laparoscopically verified acute salpingitis (AS), 299 were reviewed 2.5 to 7.5 years later. These women had all been cultured for Chlamydia trachomatis and Neisseria gonorrhoeae from the cervix. Forty-nine of 82 women with visually normal pelvic organs had also such cultures taken and were selected as controls.

In women exposing themselves to pregnancy, 50 (25.3 %) of 197 AS patients and 2 (6.7 %) of 30 control women were infertile for at least one year ($p < 0.02$). After one episode of AS, women harbouring chlamydiae, gonococci, both, or neither of these microorganisms in the cervix on admission, had the same fertility prognosis. Infertility was correlated with the number of AS episodes; the ESR mm/h at admission and the severity of the inflammatory reactions of the tubes. The use of oral contraceptives (OC) at the admission was found to be a positive prognostic factor regarding fertility. OC might protect from severe tubal inflammatory reactions.

Postinfection damage to the Fallopian tubes constitutes one of the most important causes of female infertility (1). Before the advent of antibiotic therapy, women with salpingitis and from whom Neisseria gonorrhoeae was isolated from the cervix, were reported to have a less favourable fertility prognosis, than such women, from whom gonococci were not isolated (2). Recent reports indicate, that this might no longer be true (2).

In Sweden, the annual incidence of acute salpingitis (AS), with gonococci isolated from the cervix, has decreased, whereas the annual incidence of AS in women without gonorrhoea, has increased since 1970 (1,3). It has become evident, that Chlamydia trachomatis is a more common etiological agent in AS than N. gonorrhoeae, at least in certain regions, e.g. in the Scandinavian countries (4). Clinical and laboratory differences exist between women with AS, from whom neither gonococci nor chlamydiae can be isolated from the cervix, and women culture-positive for one of these organisms or with serological evidence of chlamydial infection (5).

The present paper is a preliminary report of a follow-up study on fertility/infertility after laparoscopically verified AS, with special reference to carriage of chlamydiae and/or gonococci.

MATERIALS AND METHODS

STUDY PERIOD

The period of study was 1975 through 1979. During these years, 552 women under the age of 36 years, were hospitalized at the Department of Obstetrics and Gynecology, University Hospital, Lund, because of AS confirmed at laparoscopy (index laparoscopy). In a further 82 women, who were subjected to laparoscopy because of an assumed diagnosis of AS, the internal pelvic organs revealed no signs of inflammation or other pelvic pathology. These latter 82 women all had, however, a lower genital tract infection (LGTI).

SELECTION OF PATIENTS FOR FOLLOW-UP

Of the 552 women with AS, only those from whom specimens for the culture of C. trachomatis and N. gonorrhoeae had been obtained from the cervix, and in whom the cultures had been successfully performed, were reviewed in this study. Most of the women treated during 1975 through August 1976 were not cultured for chlamydiae. Thus excluded were 30 women, from whom cultures for neither C. trachomatis nor N. gonorrhoeae were performed and 190 women from whom chlamydial cultures had not been made or in whom the cultures were technically unsuccessful or spoiled by contaminating microorganisms. Also excluded were seven women for whom the hospital records were not found, as well as 26 women from whom no or unreliable information was obtained in the review. The remaining 299 women comprised the patient material. In all these cases, specimens for the culture of C. trachomatis and N. gonorrhoeae were obtained at the index laparoscopy and reliable information was procured in the follow-up.

SELECTION OF CONTROL WOMEN FOR FOLLOW-UP

The control women consisted of 49 of the 82 women with visually normal pelvic organs. The reasons for excluding 33 women were the same as for the AS patients, i.e. no specimens had been obtained for the culture of C. trachomatis and N. gonorrhoeae in four women, chlamydial cultures not obtained or technically unsuccessful in 25 women, hospital records lost in one case, and no or unreliable information at follow-up in three women.

DIAGNOSIS

All the patients and the control women were subjected to laparoscopy within 24 hours of admission. The inclusion criteria for performing laparoscopy as well as the criteria for a laparoscopic definition of a tubal infection was the same as in earlier studies (6).

CULTURE

Specimens for the isolation of C. trachomatis were obtained from the cervical canal using a sterile cotton-tipped swab. Cycloheximide-treated McCoy cells were used for the culturing of chlamydiae (7). Cervical, urethral and rectal specimens were cultured for N. gonorrhoeae. The culture technique and the identification procedure used for N. gonorrhoeae was that previously reported (8).

ANAMNESTIC, LABORATORY AND CLINICAL DATA

The following data were recorded at admission and used in the analysis: age, earlier pregnancies, duration of pelvic pain before admission, complaints of irregular bleeding, increased vaginal discharge, urgency and/or pain on voiding, contraceptive method used, temperature ≥ 38 °C and erythrocyte sedimentation rate (ESR) mm/h. The inflammatory changes in the tubes at laparoscopy were graded as mild, moderately severe or severe as previously described (9). Unless otherwise stated, the inflammatory changes in the tube least involved determined the classification of the patients into the three categories mentioned.

TREATMENT

The treatment during this period of time was rest in bed in hospital and antibiotics for at least ten days. The antibiotic treatment given was either 0.5 g ampicillin four times daily or 200 mg doxycycline the first day, followed by 100 mg daily. Some patients were treated with 200 mg doxycycline daily during the whole course of treatment, i.e. ten days. In a small number of patients a second drug was added, most often 400 mg metronidazole thrice daily. The physician in charge prescribed the antibiotic(s) which should be given. The treatment was started before the results of the cultures were known. All antibiotics were taken orally.

REVIEW

Questionnaires were sent to the women during the summer of 1982. Women

not answering the first questionnaire were mailed a new one two months later. The women were requested to answer questions regarding their reproductive events after the infection(s) and if they were voluntary or involuntary infertile. In the latter case, the women were asked if they had consulted a physician or not, and which examinations had been performed. The information obtained was checked against the records of the appropriate physician(s) and/or hospital(s). The women were followed up to their first pregnancy after the infection or through August 1982. The interval between the review and the diagnosed episode of AS or LGTI ranged from 2.5 years to 7.5 years with a mean of 4.0 years.

CLASSIFICATION AND SUBGROUPING OF PATIENTS

Based on the culture results from the cervical specimens obtained at the index laparoscopy, the women were subgrouped into four groups:

1. Culture positive for C. trachomatis only (C-AS)
2. Culture positive for N. gonorrhoeae only (G-AS)
3. Culture positive for both these organisms (CG-AS)
4. Culture negative for both these organisms (NCNG-AS)

In the review, the women were subdivided into:

- a. Intrauterinely pregnant (IU)
- b. Extrauterinely pregnant (X)
- c. Voluntary infertile (VOL)
- d. Involuntary infertile (INVOL)

Involuntary infertility was defined as no conception after at least one year's exposure to the chance of pregnancy. Included in the INVOL group were two women who had intrauterine pregnancy after repair of postinfection tubal changes. Unless otherwise stated, the women with extrauterine pregnancy were also included in the INVOL group. Standard infertility investigation included basal body temperature recordings, semen analyses, hysterosalpingography (HSG) and/or laparoscopy.

STATISTICAL METHODS

Chi-square with Yates' modification, contingency tables and Fisher's exact test were used.

RESULTS

COMPARISON BETWEEN AS PATIENTS AND CONTROLS INCLUDED IN, AND EXCLUDED FROM THE FOLLOW-UP STUDY

The total material of 552 women with AS had, at least 647 episodes of salpingitis up to August 1982. In 246 of the 253 AS-patients excluded from the follow-up study, the hospital records could be analysed. The 246 women excluded did not differ from the 299 AS-patients followed in the study, in the following parameters: a) age at admission, b) reproductive events before the index laparoscopy, c) duration of pelvic pain at admission, d) irregular bleedings, e) vaginal discharge, f) frequency and/or pain at voiding, g) contraceptive method used, h) ESR mm/h on admission, i) degree of inflammatory reactions in the tubes as seen at laparoscopy.

The women excluded from the study differed significantly from those included in the following ways: those excluded more often had a febrile illness ($\geq 38^\circ\text{C}$) (87/246 vs 78/299, $p > 0.02$), more often had had more than one episode of AS (49/246 vs 37/299, $p > 0.02$) and more often had gonorrhoea at the index laparoscopy (53/216 vs 46/299, $p < 0.01$).

When women culture-positive for gonococci were analysed separately, there were no differences in any respect between those excluded from, and those included in the study. The same was true for the AS-patients who were culture-negative for N. gonorrhoeae.

Of the 82 women with laparoscopically normal pelvic organs, two later took ill with AS. Both these women were at that time being treated at another hospital, and both were voluntarily infertile. No significant differences were noted, between controls excluded and included in the study, regarding the anamnestic, clinical and laboratory parameters studied. However, the women excluded from the study were more often (4/28, 14.3 %) culture-positive for gonococci than those included (2/49, 4.1 %).

INDEX LAPAROSCOPY IN THE WOMEN FOLLOWED

The age distribution of the women, subdivided according to culture results, at the index laparoscopy of the 299 AS-patients and the 49 controls, is given in Table 1.

As to age distribution, there was no difference between patients and controls. Among the AS-patients, women culture-positive for C. trachomatis and/or N. gonorrhoeae were significantly younger than the NCNG-AS patients ($\chi^2=9.26$, df 3, $p < 0.05$). The same tendency towards lower age in chlamy-

Table 1. Age Distribution of Patients with Acute Salpingitis (AS) at Index Laparoscopy and Control Women in the Various Groups Studied

Age group	C ^a		C ^b		CG ^c		NCNG ^d		Total	
	AS		Control		AS		Control		AS	
	n	n	n	n	n	n	n	n	n	n
≤ 19	30	5	12	-	6	1	42	14	90	20
20 - 24	25	5	9	-	5	-	62	5	101	10
25 - 29	14	1	7	-	-	-	42	11	63	12
30 - 36	7	-	5	1	2	-	31	6	45	7
Total	76	11	33	1	13	1	177	36	299	49

Patients infected with: ^aChlamydia trachomatis, ^bNeisseria gonorrhoeae, ^cChlamydia trachomatis and Neisseria gonorrhoeae, ^dneither Chlamydia trachomatis nor Neisseria gonorrhoeae.

dial and/or gonococcal culture-positive women was seen in the controls ($p = 0.04$).

Prior to the episode of AS, 147 (49.2 %) of the patients and 25 (51.0 %) of the controls had had at least one intrauterine pregnancy each (NS).

FOLLOW-UP

Women with one or more infections

In 274 of the 299 AS-patients, the index laparoscopy was performed during the woman's first episode of AS. Of these 274 women with first infections, 12 had one or more repeated infections during the observation period. The rate of repeated infections did not differ between women culture-positive for chlamydiae or gonococci or neither of these organisms. The influence of the number of infections on the subsequent fertility is given in Table 2.

Controls

Of the 49 women with laparoscopically normal pelvic organs, 19 were voluntarily infertile. Two (6.2 %) of the remaining 30 women were involuntarily infertile, while 28 conceived intrauterinely. The difference in fertility, between AS patients and controls, among women exposing themselves to pregnancy, was significant (50/197 vs 2/30, $p < 0.02$).

Inflammatory reactions of the tubes

The 274 AS patients with the index laparoscopy as their first episode of salpingitis, were analysed with respect to the inflammatory changes in the tubes (Table 3). Excluding women harbouring both chlamydiae and gonococci in the cervix, women in the G-AS and NCNG-AS groups had significantly milder inflammatory reactions of the tubes than women in the C-AS group ($\chi^2 = 9.54$, df 4, $p < 0.05$) (Table 3).

Women with only one episode of AS

For the analyses of the influence of positive or negative bacteriological cultures and of various clinical parameters, on the subsequent fertility/infertility, only women who had had a single episode of AS could be used. In this study, 262 such women were followed. Of these 262 women, 91 protected themselves from the chance of pregnancy (VOL-group - Table 4). Of the remaining 171 women, 133 (77.8 %) conceived while 38 (22.2 %) were involuntarily infertile (Table 4). The percentages of infertile women in the C-, G-, CG-, and NCNG-AS groups were 22.7 %, 23.5 %, 20.0 % and 22.0 %, respectively

Table 2. Intrauterinely, Involuntarily not and Voluntarily not Pregnant Women after One, Two and Three or More Episodes of Acute Salpingitis

Acute salpingitis	Pregnant			
	Intra-uterine	Involuntarily infertile	Voluntarily infertile	Total
	n	n	n	n
Once	133	38 ^a	91	262
Twice	11	9 ^b	9	29
Three or more times	3	3	2	8
Total	147	50	102	299

^a Includes two patients with intrauterine pregnancy after tubal infertility surgery. ^b Includes two women with extrauterine pregnancy. Excluding voluntarily infertile women, significantly more women with one episode of salpingitis had intrauterine pregnancy than women with two or more episodes of salpingitis ($\chi^2 = 6.82$, $p < 0.01$).

Table 3. Inflammatory Reactions of the Fallopian Tubes after One Episode of Acute Salpingitis (AS) in the Various Groups Studied

Groups	C-AS ^a	G-AS ^b	CG-AS ^c	NCNG-AS ^d	Total
Inflammatory reactions	n	n	n	n	n
Mild	37	18	8	102	165
Moderately severe	27	5	3	31	66
Severe	9	6	2	26	43
Total	73	29	13	159	274

a,b,c,d = See footnote Table 1.

Table 4. Intrauterinely, Voluntarily and Involuntarily not Pregnant Women after One Episode of Salpingitis in the Various Groups Studied

	Group	C-AS ^a	G-AS ^b	CG-AS ^c	NCNG-AS ^d
Pregnant		n	n	n	n
Intrauterine		34	13	8	78
Voluntarily infertile		26	11	2	52
Involuntarily infertile		10 ^e	4	2 ^e	22
Total		70	28	12	152

a,b,c,d = See footnote Table 1. ^e Includes one patient with intrauterine pregnancy after tubal infertility surgery.

(Table 4).

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The mean time of pregnancy exposure in the involuntarily infertile AS patients was 2.5 years (range 1 to 5.5 years). The control women had been infertile for 1.5 and 3 years, respectively.

Involuntarily infertile women examined with HSG and/or laparoscopy

In 17 AS-patients, HSG and/or laparoscopy had been performed (Table 5). Tubal pathology was found in 13 of these women (Table 5). Three of 10 spermograms from the male consorts to these women were pathologic (Table 5). None of these women had menstrual irregularities, although not all had measured BBT.

Involuntarily infertile women not examined with HSG and/or laparoscopy

Twenty-one of the remaining infertile women, with one episode of AS, had not been subjected to HSG and/or laparoscopy. In two couples the infertility investigations were stopped because of pathologic spermograms, and in three women oligomenorrhea was noted (Table 6).

Of the two infertile women in the control group, one had a short luteal phase and was treated with chlomiphene. She had not been subjected to HSG or laparoscopy. The other woman had not consulted any physician.

Involuntarily infertile women. Maximum and minimum number with tubal pathology

Excluding women with normal tubes and couples with contributing infertility factors, but including women in whom the tubes were not examined after the infection, at most 8 (19.0 %) of the 42 women in the C-AS group might have been infertile due to tubal pathology (Tables 5 and 6). The corresponding figures in the G-, CG-, and NCNG-AS groups were 3 (19 %) of 16, 1 (11 %) of 9 and 17 (18 %) of 95, respectively (Tables 5 and 6). Tubal pathology was thus, at most, a contributing factor in the infertility of 29 out of 252 women after one episode of AS (Tables 5 and 6).

Prognostic factors

The 29 women, with documented or possible tubal pathology after one episode of AS, were compared with the 133 women who had had intrauterine pregnancy after their first episode of AS, in order to detect prognostic factors. No significant differences between these two groups of women were noted regarding the age of the patient at the time of AS, earlier pregnancies, duration of pelvic pain before consulting, febrile illness and treatment given during the

Table 5. Number of Women with Hysterosalpingographic and/or Laparoscopic Evidence of Pathological Changes in the Fallopian Tubes and Contributing Infertility Factors in the Various Groups of Salpingitis Studied

Group	Tubal pathology/No. of patients examined	Contribution factors
C-AS ^a	4/4	None
G-AS ^b	2/3	Teratospermia (1)
CG-AS ^c	1/2	- " - (1)
NCNG-AS ^d	6/8	- " - (1)

a,b,c,d = See footnote Table 1.

Table 6. Infertility in the Various Groups Studied after One Infection. Maximum Possible Number of Women with Pathological Changes in the Tubes and Contributing Infertility Factors in These Women

Group	Possible tubal pathology/No. of patients examined	Contributing factors
C-AS ^a	10/10	Oligomenorrhea (1) Teratospermia (1)
G-AS ^b	3/4	None
CG-AS ^c	1/2	None
NCNG-AS ^d	20/22	Oligomenorrhea (2) Azoospermia (1)

a,b,c,d = See footnote Table 1.

stay in the hospital. Significant prognostic factors are shown in Table 7. An ESR ≤ 15 mm/h was correlated with a better fertility prognosis (Table 7). Infertility was correlated with the severity of the inflammatory changes in the tubes, as seen at laparoscopy (Table 7). A significant correlation was demonstrated only when the calculations were based on that tube, which had the least severe inflammatory alterations. The contraceptive method used at the time of the admission with AS, was correlated with the fertility prognosis (Table 7). Analyses showed, that the use of OC at the time of the AS, was correlated with a milder inflammatory reaction of the tubes and hence with a better fertility prognosis.

DISCUSSION

The women included in the present study, did in some respects differ from those excluded, i.e. the frequency of women culture-positive for gonococci, temperature $\geq 38^\circ\text{C}$ and the number of AS episodes. This is consistent with the fact that more women from the earlier period of the study were excluded, than from the later period, and the fact that the relative annual incidence of AS patients culture-positive for gonorrhea has decreased in our region, during the period of study. AS-patients with gonococci isolated from the cervix have more often temperature $\geq 38^\circ\text{C}$ than salpingitis patients without this microorganism in the cervix (5). That the number of AS episodes differed in the women included and excluded could be explained by the longer mean time of exposure, up to the time of the review, in the latter category of patients. However, the women culture-positive for N. gonorrhoeae from the cervix, who were and were not included in the study, did not differ from each other in any of the parameters studied. Neither were there any differences between women culture-negative for gonococci from the cervix in patients included and excluded from the study. Therefore, we have no reason to believe that any systematic bias was introduced by the method used to exclude patients and controls from the study population.

In our study, the patients were subgrouped according to the results of culture studies from the cervix. We want to stress that we by no means consider the tubal infection to have been caused by the organism(s) isolated from the cervix. In an earlier study (5), we classified the patients with AS, according to the results of gonococcal isolation, chlamydial isolation and the determination of micro-immunofluorescent antibodies to C. trachomatis. C-AS patients defined by these means (5) did not differ in any respect from the C-AS women as defined in the present study. Thus these patients

Table 7. Significant Differences between Intrauterinely (IU) Pregnant and Involuntarily (Invol) not Pregnant Women after One Episode of Acute Salpingitis

	IU		Invol		χ^2	p
	n	%	n	%		
EST $\leq$ 15 mm/h	54	40.6	6	20.7	$\chi^2 = 4.04$	< 0.05
> 16	79	59.4	23	79.3		
Inflammatory changes of the tubes						
mild	83	62.4	12	41.4	$\chi^2 = 7.84, df^* 2$	< 0.02
moderately severe	32	24.1	7	24.1		
severe	13	9.8	10	34.5		
Contraceptive method used						
IUD	56	42.1	12	41.4	$\chi^2 = 12.41, df^* 2$	< 0.01
OC	35	26.3	-	-		
Other or no	42	31.6	17	58.6		

* df = degree of freedom

(5), showed the same differences towards the G-AS and the NCNG-AS patients as those in the present investigation. The same similarities and differences were found among the G-AS and NCNG-AS patients in both studies.

C. trachomatis was isolated from the cervix of the AS patients twice as often as N. gonorrhoeae. This is in agreement with another Swedish study of the declining role of gonococci in the etiology of AS (3).

As in studies of LGTI (10,11), there was a correlation between the patients' age and the isolation frequency of C. trachomatis and N. gonorrhoeae.

Hedberg and Spetz (12) in 1958 reported that 40 % of women without and 33 % of women with gonococci isolated from the cervix and with AS became infertile. They did not report, however, the percentages of patients with more than one episode of AS. Furthermore, not all patients culture-negative for gonococci were treated with antibiotics in this study, while all women culture-positive for gonococci received such treatment (12).

Falk (2) in 1965 found 19 % of women who had had AS to be involuntarily infertile. These patients had generally been treated with benzylpenicillin combined with streptomycin. Half of the patients also received glucocorticoids, while half were given placebo (12). The corresponding infertility figure reported by Weström (7) in 1975 was 25 %, which is the same percentage as found in the present study.

The women in Falk's investigation (2) all had their first attack of AS. Weström et al (13) reported in 1979 an infertility frequency of 21 %, compared to 22 % in the present investigation, in patients with their first attack of AS. If women with pregnancy after infertility surgery were included in the present group, as in the above-mentioned study (13), the infertility frequency was identical, i.e. 21 %.

All cases of AS in our study were laparoscopically verified. Of Falk's cases (2), 26 % were not visually confirmed, which might mean that one third of these women did not have salpingitis but cervicitis or suffered from other conditions (6). Thus, the true infertility figure for AS patients given in that study is probably lower than indicated.

The etiologic spectrum in AS has changed since the 1960's. Thus, more than half of Falk's patients (2) had positive cultures for gonococci, while only 15 % of the women in the present study had this microorganism isolated from the cervix.

Conflicting data exist regarding the fertility prognosis after salpingitis with and without gonococci isolated from the cervix. Several studies (7,13,14) have reported a significantly better fertility prognosis for intra-

uterine pregnancy in salpingitis women with gonorrhoea isolated from the cervix. One study (2) did not find, however, any difference in that respect. In the present study, no such difference between women with C-AS, G-AS, CG-AS and NCNG-AS was found.

Several recent studies have reported an association between tubal infertility and the occurrence of serum antibodies to C. trachomatis (15,19). In our study, women culture-positive for C. trachomatis had no different fertility prognosis than women being culture-negative for this organism. The clinical picture in women with AS, from whom C. trachomatis can be isolated, is often more benign than in those AS patients not found to harbour this microorganism (20). The former women may therefore neglect their symptoms and thus not seek medical advice. This hypothesis is supported by the findings that a large proportion of infertile women with tubal pathology, diagnosed by HSG and/or laparoscopy, have antibodies to C. trachomatis often in high titers (15-19). These women often do not report any history of AS (15,16,18,19).

The fertility prognosis was found to be correlated to the degree of the inflammatory alterations in the tube, and only with the lowest degree of such changes. No such correlation was found when the calculations were based on the most severely inflamed tube. Earlier studies have reported similar findings (7,13). This supports the view that tubal pathology is the most important infertility factor after one episode of AS.

Several of the infertile women had not, and according to the answers in the questionnaire, did not intend to consult a physician because of their infertility. This contrasts with the impression from our earlier studies (7,13).

An ESR ≤ 15 mm/h at admission was a favourable prognostic sign in our study as well as in others (2,7).

The findings that different antibiotic treatment did not alter the fertility prognosis in AS women with and without gonorrhoea, might be explained by the hypothesis that not only the inflammatory reactions of the tubes are important for the fertility prognosis, but also the process of repairment (13). Also in the present study, there was no correlation between the fertility prognosis and the antibiotic drug given. One of the drugs used, i.e. doxycycline, is active and one, i.e. ampicillin, non-active against C. trachomatis in vitro (21,22).

Women using OC have a higher incidence of uncomplicated genital chlamydial infections than women with other or no contraceptive (10,23,24).

In spite of this the use of OC has been shown to diminish the risk of developing AS (1). That OC, as found in our study, might give protection from

severe tubal inflammatory reactions, has not been reported. We have no explanation, as yet, for the correlation demonstrated but it certainly adds an argument for the recommendation to use OC's for women with a high risk of acquiring STD's.

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